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PARTIAL PURIFICATION AND CHARACTERISATION OF AN RNASE FROM A FACULTATIVE THERMOPHILIC BACTERIUM

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Abstract

An RNase of molecular weight 30000 was isolated and partially purified from a facultative thermophilic bacterium, JB-1. The maximal activity was measured at pH 5,5 and 318 K for RNA digestion. The time dependence of thermal inactivation was determined at different temperatures and the activation energy of thermal inactivation calculated to be 254 kJ/mole. Ag^+, Mn^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+} and K^+ ions inhibited the RNase activity. Mg^{2+} ions had no effect on the enzyme activity. In the presence of Ca^{2+} ions an activation of RNase was observed. Phenyl-methyl-sulphonyl-fluoride and EDTA inhibited the catalytic activity. The iodoacetamide after a slight activation — also caused an inhibition of RNase activity.

Introduction

The ability of microorganisms to growth and thrive at high temperature has been a focal point of investigation. FARELL and CAMPBELL (1969) devided thermophilic bacteria into three categories: obligate or strict thermophiles with optimal growth temperatures ranging from 338 to 348 K but showing no growth below 313 to 315 K; facultative thermophiles with maximal growth temperatures between 323 and 338 K, but also capable of reproducing at temperatures below 303 K; thermotolerant bacteria with growth maxima at 318 and 323 K and showing growth below 303 K.

The question arose what is the chemical basis of the thermostability of the thermophilic or thermotolerant bacteria, i.e. how can flow the metabolism by a normal rate at higher temperature, which features result the higher thermostability of these enzymes in such microorganisms.

There are data in the literature which indicate that the lipid composition is different and as a consequence of this the membrane structures have higher melting points in thermophilic microorganisms. HEILBRUNN and BELEHRADEK (1937) pointed out that organisms growing at elevated temperatures contain lipids, with higher melting points and proposed that growth at different temperatures was dependent upon the melting point of the cellular lipids. BROCK (1967) in reviewing thermophilic growth proposed that the increase observed in percentage of saturated and branched—chain fatty acids with an increase in growth temperatures could enhance membrane stability and that the integrity of the cytoplasmic membrane may be the limiting factor in the growth of thermophiles.

Other experiments show that some individual enzymes of such bacteria are of higher conformational stability. In comparative studies, AMELUNXEN and LINS (1968

reported that nine of eleven enzymes from B. stearothermophilus were significantly more thermostable than their counterparts from the mesophile, *Bacillus cereus*.

BROCK (1967) speculated that the thermostability of thermophilic proteins might be due to a rigid, inflexibile conformation. CAMPBELL (1955) reported that the α-amylase from a facultative thermophile (*Bacillus* sp.) was thermostable if the cells were grown at 328 K, but was thermolabile from cells grown at 310 K.

BARNES and STELLWAGEN (1973) compared two thermophilic and two mesophilic enolases and found a significant relationship between an increase in thermostability and a decrease in the number of residues they considered capable of forming H-bonds. CASS and STELLWAGEN (1975) did not find any difference in the H-bonding potential comparing the phosphofructokinase of one thermophilic and two mesophilic bacteria.

In some cases the removal of Ca^{2+} ions from the enzyme resulted in loss of thermostability [FEDER (1971), VOORDOUW (1975)]. Thermophilic extracellular enzymes such as thermostable neutral protease, alkaline protease and α-amylase of Bacillus stearothermophilus, which lack disulfide bonds are stabilized by the presence of calcium. It has been suggested by POLLOCK (1962) that the calcium may take place of disulfide bonds in stabilising proteins without disulfide cross linkages.

In the frame of the comparative investigations of the conformational stability of enzymes, we have isolated a new facultative thermophilic bacterium (JB-1) and studied the characteristics some of its individual enzymes.

The present paper is concerned with some physicochemical properties of RNase from a facultative thermophilic bacterium, isolated in our department.

Materials and Methods

Growth of bacteria

JB-1 bacteria were grown up in the growth medium described by JÓNÁS (1980) with vigorous aeration at 323 K. The average yield of bacterial paste was about 3–5g wet wt. /litre of culture. The cells were harvested in the late logarithmic phase and collected by centrifugation with 6000 g for 60 min and stored as a frozen paste at 253 K.

Preparation of extract and partial purification of RNase

Bacterial paste was thawed and resuspended in 0,01 M acetate buffer, pH 5,5 and broken by treatment with lysozyme HACHIMORI (1970) or by French press. Cell debris was centrifuged off and the supernatant was saturated with ammoniumsulphate up to 60% of saturation. The insoluble proteins were removed by centrifugation and discarded. The supernatant was dialysed overnight against 0,01 M acetate buffer, pH 5,5. The dialysed supernatant was applied to a column of DEAE cellulose (15×1.4 cm), equilibrated with 0.01 M acetate buffer pH 5.5 and washed with the same buffer. Bound proteins were eluted from the column by a linear gradient of 0–0.5 M NaCl in 0.01 M acetate buffer at a flow rate of 30 ml/h. The total volume of the gradient was 100 ml and fractions of 5 ml were collected. Fractions, containing RNase activity were combined and stored at 253 K with no detectable loss of enzyme activity within 2 months.

Protein content was determined by the method of LOWRY et al. (1951) with bovine serum albumin as a standard or by measuring the absorbance at 280 nm, using an approximate extinction coeffecient of $A^{0.1\%}_{280nm}=1.0$.

Estimation of molecular weight

The molecular weight for RNase was determined by gelfiltration chromatography and sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis. The gel chromatography was

performed on Sephadex G 50 column (2.5×40 cm), equilibrated with 0.1 M acetate buffer pH 5.5 using trypsin, cytochrome c, lysozyme, soybean trypsin inhibitor and myoglobin as molecular weight markers.

The SDS-polyacrylamide gel electrophoresis was performed according to WEBER and OSBORN (1969).

Enzyme assay

The RNase activity was determined by the method of BERNARDI (1966) with some modifications. The reaction mixture contained 250 mM of acetate buffer, pH 5.5, 0.5 mg of yeast RNA and enzyme preparation in a final volume 0.5 ml. The reaction was carried out at 318 K for 20 minutes and terminated by adding 2.5 ml of 2.5% ice cold TCA solution containing 0.3 % $La(NO_3)_3$ (UDVARDY, 1973). After centrifugation the absorbancy of the supernatant was measured at 260 nm and the acid soluble digestion products were determined. One unit of RNase activity was defined as the amount of the enzyme, producing a hydrolysate having 1.0 absorbance at 260 nm in one hour reaction time.

The assay conditions were the same, when the effects of different cations, EDTA, phenyl--methyl-sulphonyl-fluoride and iodoacetamide were investigated.

Chemicals and biochemicals were reagent grade and purchased from REANAL (Budapest). Markers used for gel electrophoresis and phenyl-methyl-sulphonyl-fluoride were obtained from SERVA. Sephadex G 50 was a product of Pharmacia, Uppsala, Sweden.

Results and discussion

Partial purification of the RNase

The RNase was purified with ammonium-sulfate saturation and on DEAE cellulose column. During 60% ammonium-sulfate saturation 3 fold purification was achieved and the enzyme remained in the supernatant.

The dialysed supernatant of 60% ammonium sulfate saturation was applied to a DEAE cellulose column (Fig. 1). The RNase was not bound to the cellulose however many proteins and nucleic acids were tightly bound and were eluted only with a gradient of NaCl. During this chromatography the specific activity of enzyme

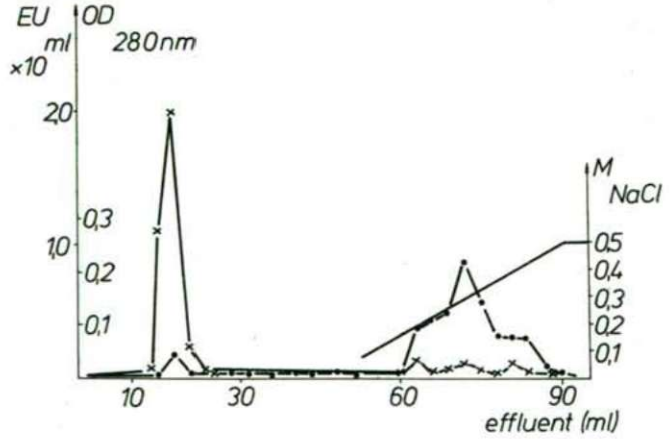

Fig. 1. DEAE cellulose chromatography of RNase. The dialysed supernatant of 60% saturation of $(NH_4)_2SO_4$ was applied to the column and eluted as described in the text. (· — ·) protein (x —x) RNase activity.

increased by 80 fold with a 79% recovery of activity. All assays, described below were carried out with this partially purified enzyme preparations. A summary of the purification procedure is given in Table 1.

Table 1. Summary of the properties of the fractions obtained during purification

		RNase activity (total units)	Protein (mg)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Crude extract		950	1453	0.653	100	1
Supernatant of 60% saturation with $(NH_4)_2SO_4$		915	468	1.95	96	3
DEAE cellulose chromatography	eluted protein fractions	57	453	0.125	6	—
	unbound protein fractions	750	14.3	52.3	79	80

The effect of temperature and pH on the partially purified RNase activity

The temperature dependence of the enzyme activity was determined between 300 and 340 K (Fig. 2). According to our results, the enzyme activity was maximal between 313 and 323 K and was assayed as a routine at 318 K. At this temperature

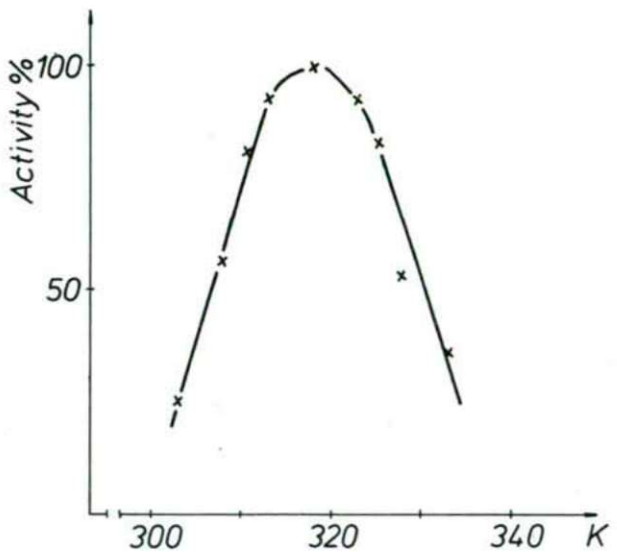

Fig. 2. Effect of temperature on the enzyme activity. In the assays 1.5 unit of RNase was used. Reaction time was 20 minutes.

the activity was proportional to the time of incubation for 15–20 minutes and was proportional to the amount enzyme.

The effect of pH on the RNase activity was evaluated in sodium acetate and tris/HCl buffers (Fig. 3). We have found, that RNase has a sharp maximum at pH 5.5.

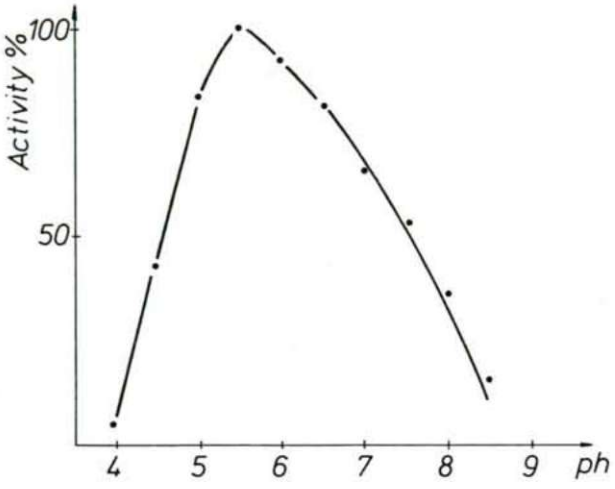

Fig. 3. Effect of pH on the activity. The assays were carried out under standard conditions, except pH which was changed as specified in the figure. The buffers used were 50 mM sodium acetate and 50 mM tris/HCl. In the assays 1.5 unit of RNase was used. Reaction time was 20 minutes.

The effect of temperature on the stability

The spontaneous inactivation of the enzyme was assayed by following the remaining activity at different temperatures at pH 5.5 (Fig. 4a). The enzyme inactivation did not follow a first order kinetic, it seemed to be composed of two parts: a fast reaction which leads to about 50% loss of the activity and a second, very slow further inactivation process. The fast period of inactivation showed a linear relationship in semilogarithmic plot (Fig. 4b). The rate constants of the denaturation process was determined from the initial slope. The logarithmus of the rate constants were plotted against the reciprocal of the absolute temperature (Fig. 5). From the linear Arrhenius plot the activation energy of the inactivation was calculated as 254.93 kJ/mole.

There are few quantitative data in the literature about the thermal stability of various RNase preparations. The kinetics of spontaneous inactivation of RNase we could not compare with mesophile ones or other thermophilic RNases, because of the lack of publications, dealing with kinetic analysis. It seems that RNases are relatively stable enzymes. According to ARIMA (1968) RNase T_2 is stable for 5 minutes at pH 6.0, 353 K, while RNase T_1 is active for 10 minutes at pH 6.0, 373 K. The heat resistance of these enzymes higher at lower pH, than in alkaline medium.

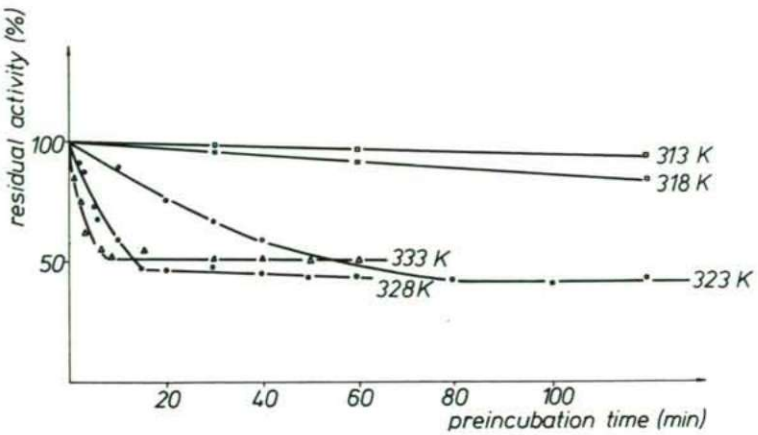

Fig. 4a. The thermal inactivation of the bacterial RNase. Enzyme solutions (0.1 mg/ml) were incubated at different temperatures. At appropriate intervals an aliquote of the enzyme solutions was taken and the reamining activity was measured.

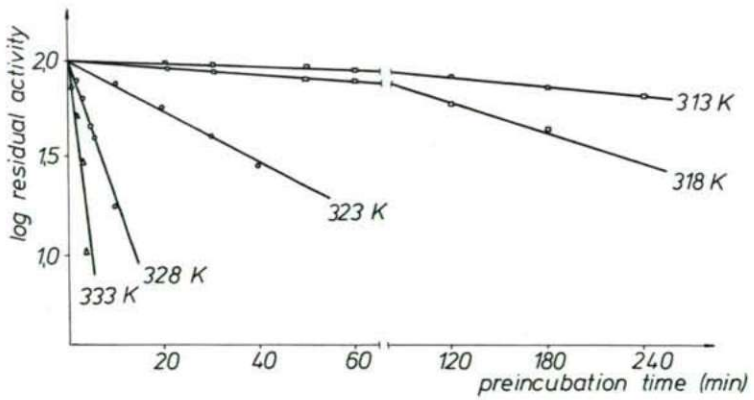

Fig. 4b. Temperature dependence of log remaining activity. Further details see text.

The thermal denaturation of RNase T_1 was determined by MOTOHISA (1979) and found $\Delta H = 564$ kJ/M. Comparing with these, the activation energy of RNase, investigated in our laboratory, seems to be not extremely high, although it was isolated from a facultative thermophilic bacterial strain.

Effect of mono and divalent cations on the activity

Generally RNases require no mono or divalent metal ions for activity. It is well known, that mono or divalent cations can considerably modify the structure of RNA molecule, which can affect the accessibility of substrate bounds to the RNase or the release of products from cleaved RNA molecules.

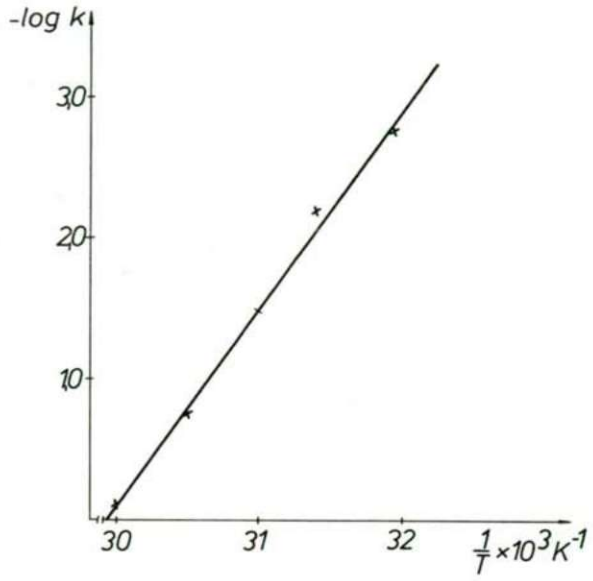

Fig. 5. Temperature dependence of the rate constants of thermal inactivation of RNase. The enzyme solutions were incubated in 50 mM; pH 5.5 of acetate buffer.

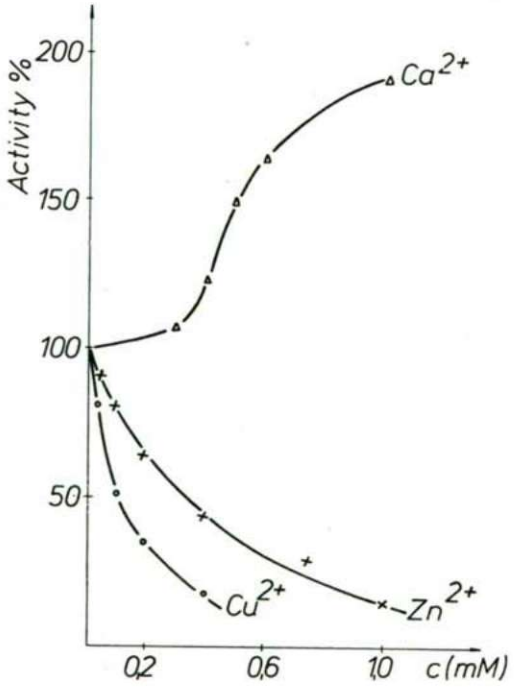

Fig. 6. Effects of Zn^{2+}, Cu^{2+} and Ca^{2+} on the activity of bacterial RNase. Enzyme solution contained 0.2 mg/ml of proteins.

Table 2. Effect of mono and divalent cations on the enzyme activity. Enzyme solutions (0.2 mg/ml containing different concentrations of various cations were incubated at 318 K, at pH 5.5 for 20 minutes and the remaining activity was measured.

Metal	Concentration mM	Inhibition (%)
Mn^{2+}	0.1	19.6
Co^{2+}	0.02	16.5
	0.75	24.4
	1.0	35.9
Mg^{2+}	0.1	no inhibition
	0.2	no inhibition
Ni^{2+}	0.2	23.4
	0.4	43.4
K^{+}	0.04	0.0
	0.1	0.0
	0.2	9.8
	0.4	19.2
Ag^{+}	0.2	17.1
	0.4	39.3

The effect of various cations on RNase activity are presented in Table 2, and Fig. 6. Almost in all cases inhibition of activity was observed at concentrations below 1 mM. Mg^{2+} ions did not effect the reaction rate. In the presence of Ca^{2+} ions the enzyme activity increased. Enhanced activity was found below 1 mM concentration. Further increase of the Ca^{2+} concentration had no further activating effect (not shown on the figure).

Molecular weight

The molecular weight of RNase was 30900 as estimated by Sephadex gel filtration. The measurements, using SDS polyacrylamide gel electrophoresis gave similar results a molecular weight of 30000.

The molecular weight of microbial RNases varying from 10000 to 40000. Molecular weight of RNase of Bacillus cereus was found 30000. The RNases isolated from Aspergillus oryzae and Ustilago sphaerogena had a molecular weight of 10000.

Effect of specific chemical reagents on RNase

The carboxamidomethylation of RNase at pH 5.5 — after a slight activation — measured at about 10^{-8} M concentration of reagent — caused inhibition of activity. The carboxamido-methylated residue might have been essential for the catalytic activity (Fig. 7).

In some fungal RNases activation was observed after pretreatment with iodoacetic acid or iodoacetamide (ARIMA, 1968). The effect of phenyl-methyl-sulphonyl-

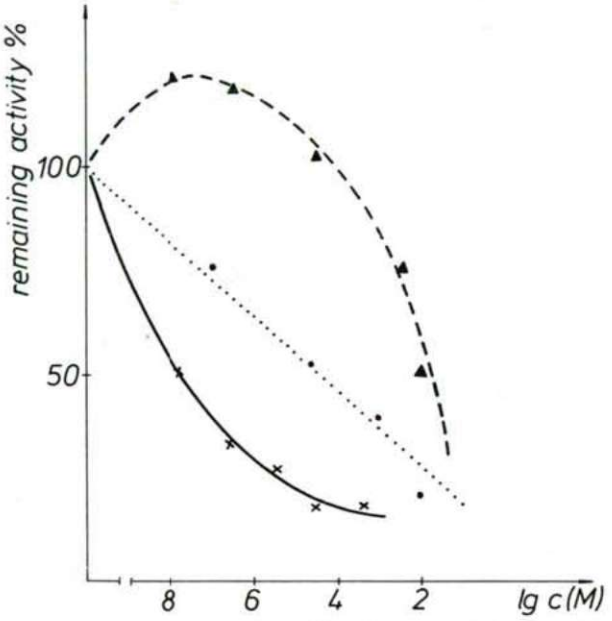

Fig. 7. Effect of phenyl-methyl-sulphonyl-fluoride (PMSF), iodoacetamide and EDTA on RNase activity. Assay conditions were those described in the Methods section. (x—x PMSF; △——△ iodoacetamide; ○·····○ EDTA).

fluoride was determined at concentration from 10^{-8} to 10^{-2} M. The phenyl--methyl-sulphonyl-fluoride inhibited the enzyme activity, which suggest that seryl side chains may have important role in the mechanism of the catalysis.

The RNase activity was totally abolished by the addition of EDTA. This indicate, that this enzyme is an RNase of metalloprotein type.

The data presented show that the facultative thermophilic bacterial RNase has properties similar to B. cereus RNase in respect to inactivation by EDTA and molecular weight RUSHIZKY (1964) and Ca^{2+} activation SHIIO (1966).

The properties of enzyme were also similar to RNase of Rhizopus in respect to stability and inhibition by mono or divalent cations, however this latter enzyme was not inhibited by EDTA (TOMOYEDA et al. 1969).

As above detailed experimental results show our RNase preparation of JB-1 bacteria behaves "uniformly" towards the inhibitors, i.e. all inhibitors caused practically complete loss of enzyme activity and no heterogenity could have been indicated. The apparent heterogeneous behaviour of the enzyme preparation in the heat inactivation experiments (Fig. 4) might reflect the existence of two form of different stability of one enzyme, or the existence of two enzymes with similar active site but with different conformational stability. If only one enzyme exists in the bacteria, either this enzyme could occur in two conformational (or quaterner structural) forms of different stability and different catalytic activity or the enzyme occurs under normal conditions in a more active but less stabile form and the heat treatment causes its transformation to a less active but more stabile form. The further investigation of the problem is in progress in our laboratory.

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HYDROECOLOGY OF THE VEGETATION OF SANDY FOREST-STEPPE CHARACTER IN THE EMLÉKERDŐ AT ÁSOTTHALOM

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(Received August 30, 1981)

Abstract

One of the most intact areas of the ancient land unit of sandy forest-steppe character in the southern part of the region between the Danube and the Tisza is the conservation area of Emlékerdő at Ásotthalom about 30 km west of Szeged. This area has even been exempted from grazing. It was placed under protection many decades ago. Consequently, its plant communities land themselves particularly well for performing hydroecological investigations.

1. In the open steppe grass stand of *Festucetum vaginatae* on the humus-poor and quick sand hills and ridges, the subassociation of Stipa borysthenica dominates today. Its character species belong to the components 2 and 3 of steno-xerophytes.

2. In the spaces among the drying sand-hills of humus sandy surface soil, three sub-associations of *Festuco rupicolae-Salicetum rosmarinifoliae* can be differentiated. The species of their closed grass stands belong to steno-xerophyte 3 and asteno-xerophyte 1 categories on the basis of their contributions to coverage.

3. The differentiation of the subassociations of *Festuco (Querco)-Populetum albae* on humus sandy surface soil with buried brown forest soil seems to be related to the water and nutrient supply of the humus layer near the surface.

The regulation of internal waters resulted in the gradual sinking of the underground water level. Today the white and grey poplar stands are no more able to regenerate and are doomed to slow extinction. It is evident that the saving of the protected area is a very important task.

Introduction

The nature conservation area called Emlékerdő at Ásotthalom is one of the oldest protected areas in the southern part of the Great Hungarian Plain between the Danube and the Tisza. This ancient sandy forest-steppe relict (KISS 1915) left untouched for about a century and exempted even from grazing proved to be very suitable for the biocenotical and ecological investigation of the fauna (CSIZMAZIA 1979) and flora (BODROGKÖZY 1957) of the sandy forest-steppe of the Plain. A report on the composition, soil ecological conditions of the grass stands and poplar forests covering its sand-hills and the spaces between the hillocks was published 25 years ago ( ). In this paper the results of investigations performed earlier were also summarized. The aim of the present study was to explore the hydroecological properties of the phytocenoses in this protected area by the application of improved methods and register the changes which have taken place in the last ten years.

BABOS's work (1955) deals with the formation of the sandy forest-steppe in the region between the Danube and the Tisza and the forest stands there. The diversification of the relief in this area is due to the fact that the Danube had retreated from east to the west in geological times and the great mass of fluviatile sand it left behind was capriciously rearranged by the wind. The underground water table located once at a higher level resulted in the formation of bogs in the spaces between the sand-hills. This caused the sharp differentiation of the vegetation on the sand-hills from that on the wind-formed ridges. For the favourable water supply, the ancient forest stands of the sandy forest-steppe were made up of gallery forests

of *Convallario-Quercetum roboris* and *Festuco-Quercetum roboris*. The unfavourable environmental effects produced partly by bogs characterizable by helophytes and partly by sand ridges covered with the open stands of xerophytes of sandy steppe, prevented the developing of continuous forest stands.

These ancient oak-forests have disappeared not only from the environs of Szeged, but are also very seldom found in the Great Plain. The becoming more arid of the climate, and the intensified regulation of internal waters during the last century have caused the lowering of the underground water level. The perished oak stands and those ones which fell victim to the increasing penury of timer in the plains in the course of historical times, were replaced by *Populus alba* and *P. canescens*. By vegetative propagation these have survived as well as some of the characteristic representatives of the herb and shurb strata of the oak stands. The responses of some sandy-steppe phytocenoses to increasing dryness of soil as well as the changes in species composition of these phytocenoses can be evaluated only in the knowledge of the hydroecological demands resp. tolerance of each species. These values for the characteristic species of the single stands can be determined in the same way as the contributions of species belonging to a particular hydroecological category to total coverage. Graphs plotted on the basis of the values further the better understanding of these relationships.

Materials and Methods

In the nature conservation area of sandy forest-steppe type at Ásotthalom, the sociological and hydroecological conditions of three associations and their smaller subunits were investigated with special regard to those physical and chemical parameters of the soil which influence principally the seasonal changes of the subunits.

In this paper only the results obtained during 1980 will be presented.

The distribution of soil fractions should be regarded as one of the most important influencing factors. For fractionation of soil the hydrometer method was used and complemented with sieving. The values for organic matter content, calcium carbonate content and the hy value were determined in early summer and late summer. Soil moisture was also measured.

The scales of ELLENBERG and ZÓLYOMI et al. for the expression of moisture demand and tolerance are commonly used in our country (Soó, 1964—80). This system contains ten categories and also a group of indifferent species. The hydroecological data collected and evaluated during the phytocenological and synecological investigations in the Great Plain for about 30 years alloved a more detailed hydroecological categorization of the species of the single phytocenoses. It was considered reasonable to eliminate the group of indifferent species, since each of its representatives can be listed into a suitable category. The single groups were marked with the abbreviated forms of their Latin names instead of numbers.

The new classification is the following:

1. hd (hydatophytes) species growing submersed or floating on the surface of standing and slowly flowing waters as well as species attached to some firm substrate.
2. hhe (hydato-helophytes) species growing in the littoral.
3. he (helophytes) species in damp, temporarily flooded habitats.
4. hhg (helo-hygrophytes) species of very humid, marshy, silty habitats.
5. hg (hygrophytes) species growing in moist environment.
6. mhg (meso-hygrophytes) species inhabiting slightly damp habitats.
7. m (mesophytes) species growing in medium dry situations.
8. xm (xero-mesophytes) species in drying habitats with a wide spectrum of hydroecological adaptability.
9. ax (asteno-xerophytes) species growing on dry soils.
10. sx (steno-xerophytes) species living in very dry situations (Species of low competitiveness on sand-hills, rocky slopes and loess ridges).

Further improving of the system was thought justified for the transitions. In the interest of that three sub-types were differentiated in each category. These were marked with figures. In this way, by using 28 sub-types, it was easy to solve the problems emerged (1 = transition to the previous category, 3 = transition to the next category).

For the identification of a particular species, it is very useful to plot the hydroecological graph of the species.

On the basis of literary and own data, namely, it is possible to determine the minimum and maximum points of the graphs for each species. The higher the percentual value for the maximum point, the nearer the minimum points to one another, i.e. the more characteristic the species from the aspect of hydroecology (their tolerance interval is narrow). In addition to the hydroecological characteristics for the single species, those for a particular phytocenosis can also be determined by means of the plotted graphs for the single species components. The complex graph can be plotted partly on the basis of species belonging to the single categories, partly on that of their contributions to cover.

It is desirable, however, to determine also the moisture content of the soil of each phytocenosis parallel with categorization. Differently from the practice adopted in the past when conclusions were drown on the basis of a single analysis, during these studies it was found more useful to take into account also the moisture content of the soil and express it in the percentage of wet resp. dry soil. These values were finally converted to lit dm^{-3}. In these analyses undisturbed sampling conditions were maintained. These three values for moisture content will be even more suggestive if in their graphical illustration their ratios are also considered (Fig. 3).

Discussion

Cenosystematical categorization of plant associations on the sandy forest-steppe of the protected area Emlékerdő (Soó, 1956, 1965–1980):

FESTUCO-BROMEA Jakucs 67

Festucetea vaginatae Soó 57

Festucetalia vaginatae Soó 57

Festucion vaginatae Soó 29

Festucetum vaginatae (Rapcs. 23) Soó 29 *danubialae* Soó 29

— — festucetosum vaginatae (typicum) Magyar 33

— — stipetosum borysthenicae (=sabulosae) (Kern 1863) Soó 39

— — populetosum albae-stoloniferae Soó (29) 39

Festucetalia valesiacae Soó 57

Festuco (rupicolae) — Salicetum rosmarinifoliae (Magyar 33) n.n.

(Syn.: Festucetum vaginatae salicetosum rosmarinifoliae)

— — festucetosum vaginatae (n.n.)

— — festucetosum rupicolae (typicum, n.n.)

— — poetosum angustifoliae (n.n)

— — molinietosum coeruleae (n.n.)

QUERCO-FAGEA Jakucs 67

Quercetea pubescentis-Petraeae (Oberd. 48) Jakucs 60

Quercetalia pubescentis Br-Bl. 31

Aceri tatarico-Quercion Zólyomi et Jakucs 63

Festuco (Querco)-Populetum (Soó n. prov. 71) Bodrk. (57) 81

— — calamagrostetosum (n.n.)

— — festucetosum rupicolae (typicum, n.n.)

— — salicetosum rosmarinifoliae (n.n.)

Structure and synecology of the single associations and their sub-units (Fig. 1).

Festucetum vaginatae (Rapcs. 23) Soó 29/39 *danubiale* Soó 29/39.

The fluvial sand deposited and later left behind by the Danube retreating from the eastern part of the plain to the west may have become eolitic after drying and

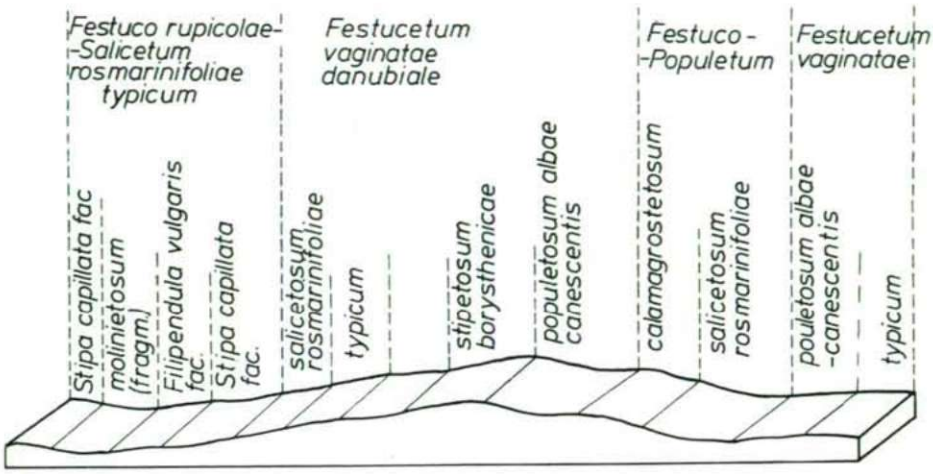

Fig. 1. Zonal distribution of plant communities in the area of investigation.

was delivered to shorter or longer distances by the wind. After several rearrangements, it formed hills, barkhans (BABOS, 1955). During this process, the coarse fraction separated out and the sand mass arriving to Szeged was of medium fine fraction. In the western areas of the region between the Danube and the Tisza, the coarse sand fraction predominates principally (SZABÓ, 1973). In our area, just as in the other sandy areas of our country, its stands occur on the sand-hills and higher sand ridges forming there open grass associations (HARGITAI, 1940; ZSOLT, 1943 ZÓLYOMI, 1958; BORHIDI, 1956; BODROGKÖZY, 1956, 1957; SIMON, 1962; PRÉCSÉNYI, 1961, 1963; SZODTFRIDT et al., 1968; KOVÁCS-LÁNG and SZABÓ, 1971), in which the moss-lichen synusium can assume a dominant role. VERSEGHY and KOVÁCS-LÁNG (1974) studied the structure and production of this association. These studies were concentrated only on the herb stratum.

It has more than one subassociations on the higher sand-hills and sand ridges at Ásotthalom:

F. v. festucetosum vaginatae (typicum) MAGYAR 33

A quarter of a century ago this was the dominant steppe grass variety. Today it is found only on the lower ridges. The distribution of its species according to the hydroecological categories is the following:

Though the species belonging to the subgroup of typical steno-xerophytes (sx2) exceed the sx3 species in respect of species number, evaluation of their contributions to the coverage points to the advantage of the latter ones (Table 1). During the last quarter of a century, *Stipa capillata* of the sx2 type has become gradually facies-forming together with ax3 *Calamagrostis epigeios*. The latter has two ecotypes: the asteno-xerophyte type growing on dry habitats and the hygro-mesophyte (hg1) one occurring in more humid environment along the river. Its occurrence in clearing vegetation is only transitory.

The xero-mesophytes exhibit great species numbers and low D values. These belong mainly to subgroup xm3, and their adaptability is greater than that of xm1 Scabiosa ochroleuca, Galium verum etc. (Table 1).

Table 1. Festucetum vaginatae danubiale stipetosum borystenicae (1), festucetosum vaginatae (2) populetosum albae (3)

	Subass.:					1	2	3
	Steno-xerophyta:							
H Festucion vaginatae	*Festuca vaginata*	sx3	F 2	T 4	N 1			
H Festucion vaginatae	*Stipa borysthenica*	sx3	F 2	T 3–4	N 1			
H Festucion vaginatae	*Euphoria seguieriana*	sx3	F 1–2	T 3	N 1			
H Festucion vaginatae	*Tragopogon floccosum*	sx3	F 1–2	T 4	N 2–3			
H Festucion vaginatae	*Alkanna tinctoria*	sx3	F 1	T 4–5	N 1			
N Festucetalia vaginatae	*Fumana procumbens*	sx3	F 1–2	T 4	N 1			
H Festucion vaginatae	*Dianthus diutinus*	sx3	F 1–2	T 4	N 1			
G Festucetalia vaginatae	*Carex liparicarpos*	sx2	F 2	T 4	N 2			
H Festucion vaginatae	*Stipa capillata*	sx2	F 2	T 3–4	N 2			
Ch Festucion vaginatae	*Alyssum tortuosum*	sx2	F 2	T 3–4	N 1			
H Festucion vaginatae	*Koeleria glauca*	sx2	F 2	T 3	N 2			
H Festucion vaginatae	*Centaurea arenaria* ssp. *tauscheri*	sx2	F 2	T 4	N 1			
H Festucion vaginatae	*Syrenia cana*	sx2	F 2	T 4	N 1			
H Festucion vaginatae	*Onosma arenaria*	sx2	F 1–2	T 4	N 1			
Ch Festucion vaginatae	*Dianthus serotinus*	sx2	F 1–2	T 4	N 1			
Ch Festucetalia valesiacae	*Thymus marschallianus*	sx1	F 1–2	T 3	N 1			
Th Festucion vaginatae	*Salsola kali* ssp. *ruthenica*	sx1	F 1–2	T 3	N 0			
G Festucetalia valesiacae	*Equisetum ramosissimum*	sx1	F 2	T 3–4	N 0			
Th Bromion tectorum	*Secale silvestris*	sx1	F 1–2	T 4	N 1			
	Asteno-xerophyta:							
H Populetalia	*Calamagrostis epigeios*	ax3	F 2–3	T 3	N 3			
Th Festuco-Brometea	*Verbascum lychnitis*	ax3	F 2	T 3	N 2–3			
H Festuco-Brometea	*Linaria genistifolia*	ax3	F 2	T 4	N 2			
Th Festucion vaginatae	*Polygonum arenarium*	ax2	F 1–2	T 4	N 1–2			
Th Bromion tectorum	*Tragus racemosus*	ax2	F 2	T 4–5	N 2			
Ch Festucetalia valesiacae	*Artemisia campestris*	ax1	F 2	T 2	N 2			
G Festuco-Bromea	*Cynodon dactylon*	ax1	F 2	T 3–4	N 3			
Th Festucetalia valesiacae	*Minuartia glomerata*	sx1	F 1–2	T 4	N 1–2			
Th Festucion vaginatae	*Alyssum tortuosum*	sx1	F 2	T 3–4	N 1–2			
H Festuco-Brometea	*Phleum phleoides*	ax1	F 2	T 3	N 2			
H Festucetalia vaginatae	*Silene otites* ssp. *pseudotites*	ax1	F 2	T 3	N 2			
Ch Festuco-Brometea	*Teucrium chamaedrys*	ax1	F 1–2	T 2	N 2			
H Festucetalia valesiacae	*Minuartia verna*	ax1	F 1–2	T 0	N 1–2			
Ch Festucetalia valesiacae	*Alyssum montanum* ssp. *gmelini*	ax1	F 2	T 3	N 1			
H Festucetalia valesiacae	*Chrysopogon gryllus*	ax1	F 2	T 3–4	N 2			
	Xero-mesophyta:							
H Festucetalia	*Poa angustifolia*	xm3	F 3	T 0	N 3			
Th Festuco-Bromea	*Arenaria serpyllifolia*	xm3	F 2–3	T 0	N 2–3			
H Festuco-Brometea	*Eryngium campestre*	xm3	F 1	T 4	N 2–3			
H Festuco-Brometea	*Scabiosa ochroleuca*	xm3	F 1–2	T 3–4	N 1			
Th Festuco- Brometea	*Odontites lutea*	xm3	F 2	T 4	N 1–2			
H Festuco-Brometea	*Euphorbia cyparissias*	xm3	F 1–2	T 0	N 0			
H Festucetalia valesiacae	*Astragalus onobrychis*	xm3	F 1	T 3	N 1			
H Festucion vaginatae	*Chondrylla juncea*	xm3	F 2–3	T 4	N 2			
Th Festucetalia valesiacae	*Medicago minima*	xm3	F 1–2	T 1–2	N 2			
Th Festuco-Brometea	*Erophila verna*	xm3	F 2–3	T 0	N 1–2			
Th Festuco-Brometea	*Calamintha acynos*	xm3	F 1–2	T 2–3	N 2			
Th Chenopodio-Scleranthea	*Camelina microcarpa*	xm3	F 2–3	T 3	N 2–3			

	Subass.:					1	2	3
Th Secalietea	*Crepis rhoeadifolia*	xm3	F 2–3	T 4	N 2–3			
H Festucetalia valesiacae	*Dianthus pontederae*	xm2	F 2	T 4	N 2			
Th Secalietea	*Consolida regalis*	xm2	F 2	T 3	N 2			
G Festucetalia valesiacae	*Iris humilis* ssp. *arenaria*	xm2	F 2	T 3	N 1–2			
H Festuco-Brometea	*Galium verum*	xm1	F 0	T 2–3	N 1–2			
Th Festuco-Brometea	*Holosteum umbellatum*	xm1	F 2	T 4	N 2–3			
M Populetalia	*Populus alba*	xm1	F 2–3	T 4	N 1–2			
M Populetalia	*Populus canescens*	xm1	F 2–3	T 4	N 1–2			

Signes used (D%)

25–50
10–25
5–10
1–5

F.v. stipetosum borysthenicae (KERN, 1863) SOÓ 39 corr. n.

It is found on the dryest sand-hills and sand ridges in our area. During the last decades it has gained ground considerably (BODROGKÖZY, 1957). This is likely to be related to the more intensive drying of sandy areas as a consequence of the operation of the canal systems in the interest of regulating internal waters.

Seasonal changes of its environmental factors

In the organis matter-poor, medium fine sandy soil of its stands (Fig. 4) the clay-silt fraction is minimal: its ability to bind precipitation is small. Studies performed by SZABÓ (1973) are also in support of that. The small difference between the courses of the graphs for soil moisture expressed in the percentages of dry and wet soil weight throughout the vegetation period also indicates that. At the same time, in the vernal aspect, precipitation is abundant, the number of sunny hours of the day decreases (during 20 days before the examination 64 mm: Fig. 2, 3).

Despite repeated atmospheric precipitation in early summer, 1980, the water regime appeared to be unfavourable, but for the low dead water content, the grass stand was still green. By the end of August, however, the value for soil moisture near the surface was only 0.01 lit dm^{-3} and the vegetation was discoloured.

Hydroecological characterization of its species

The number of sx species is greater relative to the type; concerning contribution to coverage, *Stipa borysthenica* dominated. Its gaining ground at the expense of *Festuca vaginata* seems to be related to the increasing drying of the environment (Fig. 5).

Similarly to the type, the number of xero-mesophytes with wider ecological adaptability is greater, but their total contribution to cover is smaller. These belong mostly to xm3 subgroup and have greater rooting depths. Such are e.g. *Chondrilla juneca, Eryngium campestre* etc. Of the therophytes only those survived, which bloom under the more favourable hydroecological conditions of the vernal aspect, as *Arenaria serpyllifolia, Erophila verna, Medicago minima,* etc.

The 19 stenoxerophytes are species of Festucion vaginatae from the viewpoint of cenosystematics, apart from a few exceptions. One of the most important members

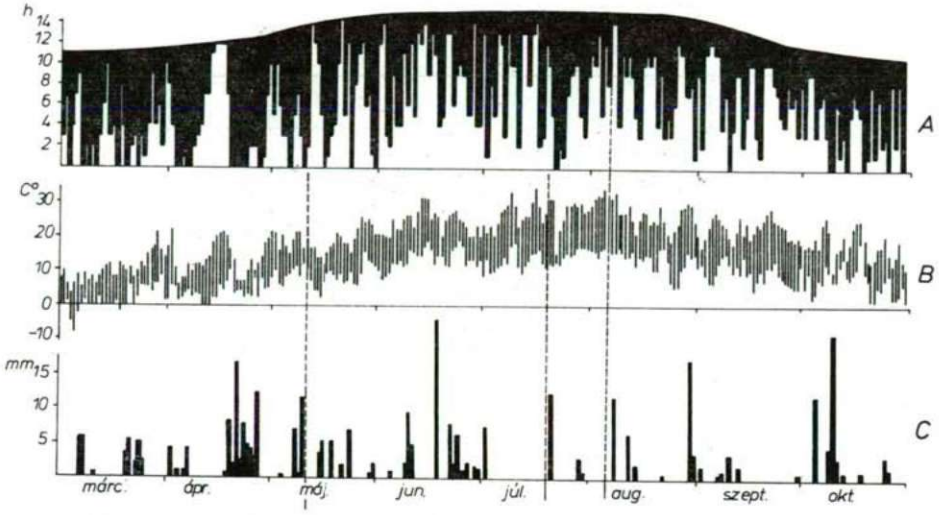

Fig. 2. Diel rhythm of the components in the vegetation period of 1980.
A: Distribution of sunlit and cloudy hours, B: Daily maxima and minima of air temperature, C: Precipitation in March, April, May, June, July, August, September and October.

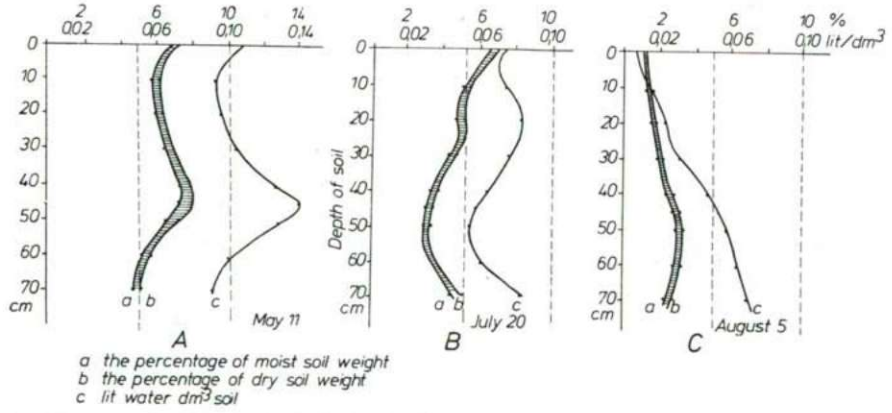

Fig. 3. Changes of moisture dynamics in the humus-poor quick sand profile of hill top soil in the vernal resp. aestival aspect in 1980.
a: the percentage of moist soil weight; b: the percentage of dry soil weight; c: lit water dm^{-3} soil.

of this subassociation from the aspect of nature conservation is *Dianthus diutinus* the endemic species of the sandy steppes between the Danube and the Tisza. Recently it has diminished considerably.

F. v. populetosum albae-canescentis (Soó) 29/39

The two poplar species *Populus alba* and *P. canescens* can even ascend the lower sand-hills and sand ridges by means of their sprouts. For the unsufficient nutrient and moisture supply, however, they can only form shrubberies there. Owing to

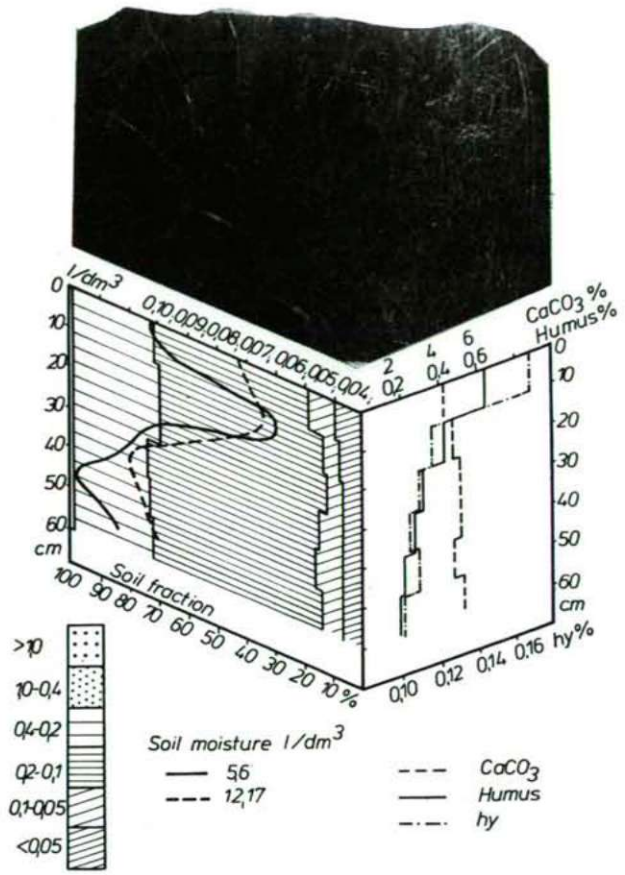

Fig. 4. Physical, chemical and water dynamical conditions in the soil profile of the plant community on sand hill top in 1980.

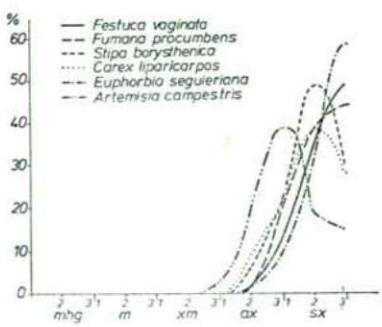

Fig. 5. Hydroecological graphs for the species components of open sandy steppe
mhg: mesohygrophyton, m: mesophyton, xm: xeromesophyton, ax: astenoxerophyton, sx: stenoxerophyton.

their shading effect several such species appear which were missing from the former two subassociations, or occur only sparsely there as Poa angustifolia and the facies-forming ax3 Calamagrostis epigeios (Table 1). The latter one has increased in the last quarter of a century and contributed to the further drying of the habitat. Analytical data pertaining to the condition of its soil are known (BODROGKÖZY, 1975).

Festuco rupicolae-Salicetum rosmarinifoliae (MAGYAR 33) n.n.

Before the regulation of internal waters, the underground water table lying higher in the sandy areas of the region between the Danube and the Tisza complemented with the accumulating stagnant waters resulted in the formation of marshes between the sand-hills. The stands of *Molino-Salicetum rosmarinifoliae* (MAGYAR 33) Soó 57 made up of Molinion and Agrostion components dried out gradually as a consequence of the lowering of underground water level, giving place for the species of Festucetalia and Festuco-Brometea.

The oxidation of humus in its soil as well as the secondary sand cover in some places, caused the species of Festucetalia, moreover those of Festucion vaginatae to became dominant in the higherlying places between the sand-hills. In this way another independent association formed between *Festucetum vaginatae* and *Molinio-Salicetum rosmarinifoliae* (MAGYAR, 1933). Its two character species *Holoschoenus romanus* and *Salix repen*-ssp. *rosmarinifolia* cannot be regarded as characters pecies of Festucetum vaginatae. The type is linked with the (above mentioned) adjacent associations by subassociations of transitory nature. It is also likely to be connected with *Astragalo-Festucetum (sulcatae) rupicolae* (BODROGKÖZY, 1957).

Its soil ecology

It is particularly its more favourable organic matter content and water supply relative to the former one, that assure better conditions of life which is evident first of all in the vernal aspect (Fig. 6). Its single subassociations, however, essentially differ from one another:

F.(r). — *S.r.* festucetosum vaginatae (n.n)

(Syn.: Festucetum vaginatae salicetosum rosmarinifoliae (Magyar 33) Soó 39).

It is a transition to Festucetum vaginatae, forming a zone at higher levels of the spaces between the sand-hills (Fig. 1). During the last decades, it has spread very greatly in our area. It is, however, well separated by *Salix rosmarinifolia* and *Holoschoenus romanus*.

Hydroecological position

In respect of the distribution of species, sx2 type species have become dominant instead of sx3 components within the category of stenoxerophytes. Type sx2 *Stipa capillata,* sporadically *Chrysopogon gryllus* occur in large numbers and with increasing frequency. The xeromesophytes are still crowded into the background (Table 3, Fig. 11). The hydroecological plots of some character species also show the transitory nature of this subassociation (Fig. 6).

F(r)–S.r. festucetosum rupicolae (typicum) n.n.

In the Danube–Tisza midregion, and thus also in the protected area of Emlékerdő, the impoverished meadow communities between the sand-hills in the sandy forest-steppe separate into well distinguishable facies. Their occurrence shows cor-

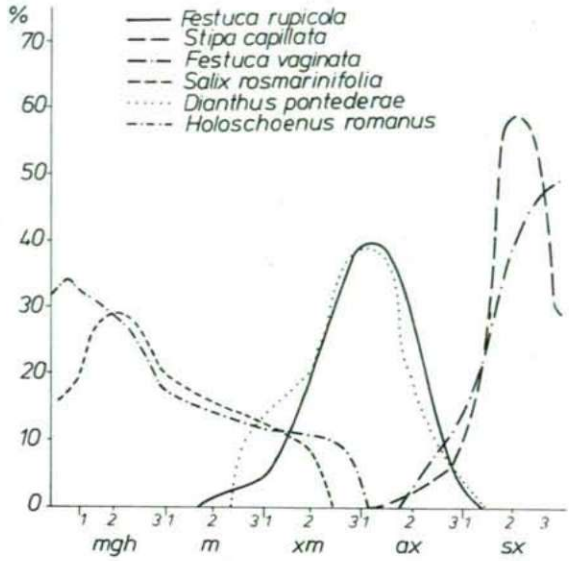

Fig. 6. Hydroecological graphs for the character species of a transitional stand of *Festuca vaginata* in the meadow between sand-hills.

relation with the seasonal changes of the water regime in the vegetation period. The thing they have in common is that after the favourable moisture supply during spring a very dry period follows in summer during which the water content of soil in their rooting zone is less than 0,05 lit dm^{-3} (Figs. 7, 8).

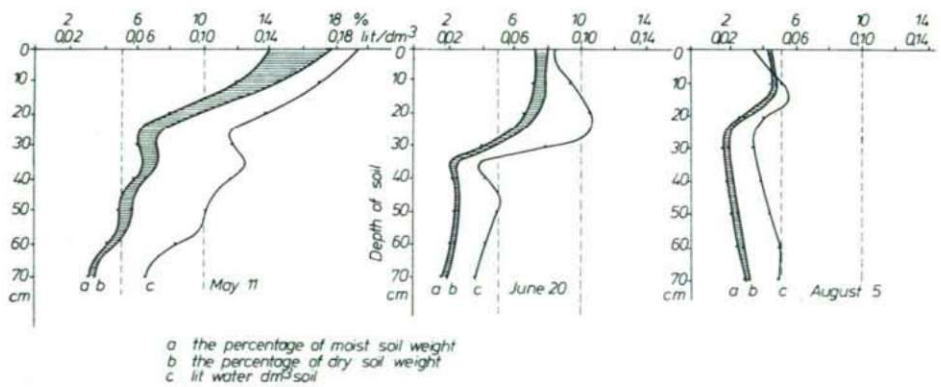

Fig. 7. Changes of moisture content in the humous sandy soil profile of typical meadov plant community in the space between sand-hills.
a: the percentage of moist soil weight; b: the percentage of dry soil weight;
c: lit water dm^{-3} soil.

Stipa capillata facies

This is closest related to the previous subassociation in respect of species composition, but the contribution of Salix repens ssp. rosmarinifolia to cover is essentially greater. The water regime of its soil is much better than that of the previous facies.

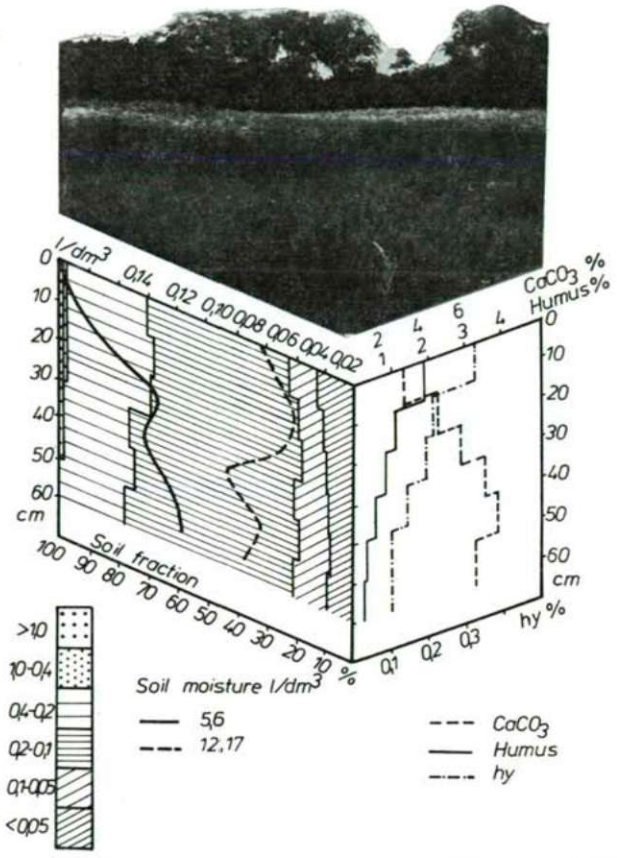

Fig. 8. Physical and chemical properties of the soil profile between sand-hills as well as the maximal and minimal values of its water budget in 1980.

It can be characterized by a more favourable organic matter supply, which is due partly to the higher phytomass production (Table 4) and decomposition and partly to the excellent water-binding capacity of humus carried down from the sand-hills by the wind. Here the possibilities of binding resp. storing atmospheric precipitation are better. In the surface-near soil layers, twice as much water was demonstrable than in the neighbouring hill top in its vernal aspect in 1980. The water regime in deeper soil layers is, however, more balanced (Fig. 7). This facies is the poorest one of the subassociation. Its character species are sx2 *Stipa capillata,* ax1 *Festuca rupicola* as well as *Poa angustifolia, Potentilla arenaria, Stachys recta* of the xm3 type each and *Medicago falcata* and *Linum austriacum* both belonging to the type xm2. Species of the mhg type *Salix repens* ssp. *rosmarinifolia* and *Polygala comosa.*

Filipendula vulgaris facies

It is found in the richest sections of the former marshy meadows among the sand-hills, in the northern littoral zone of windformed ridges, where the shading effect of nearby poplar stands prevails. In the summer period ot is much better

protected against the damaging effect of insolation. Its stands are completely closed with double herb stratum. Organic matter content of its soil is higher than that of the former facies (Fig. 10). Its water regime is better, but the root effect of the nearby poplar stands prevails in a considerable measure particularly during summer (Fig. 9).

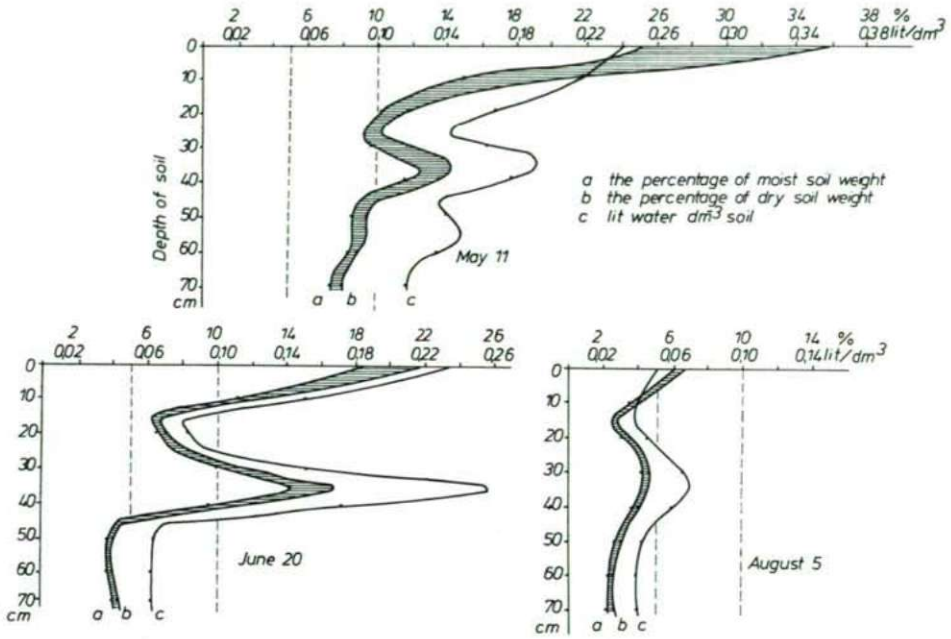

Fig. 9. Changes of soil moisture dynamics of the *Filipendula* facies of the typical meadow among sand-hills.

Of the species of Filipendula vulgaris facies, however, only those could survive, which tolerated the unfavourable water supply during summer for their wider adaptability. These belong principally to the category of xeromesophytes, as xm2 *Salvia pratensis, Iris humulis* var. *arenaria;* xm1 *Filipendula vulgaris, Galium verum, Knautia arvensis* ssp. *arvensis.*

Members of Molino-Arrhenatheretea are m3 *Tragopogon orientalis* as well as *Lotus corniculatus* and *Ranunculus acris* of the m2 type both. Moreover, in some places as species of Molinietalia, the hygrophyte *Thalictrum simplex* v. *galioides* and the characteristic name-giving species of the ancient marshy meadows in the spaces between hillocks *Molinia coerulea* are also observable occurring by stems (Table 2).

The percentual values for the contributions of species belonging to the single hydroecological categories to coverage in comparison with similar values for *Festuca vaginata* subassociation are shown in Fig. 11. It is seen that the plotted graph for species components of *Festuca rupicola* subass. culminates in the ax1 subtype which forms a link between xero- and astenoxerophytes, while in the case of the subass. *Festuca vaginata* at sx2 (Fig. 11).

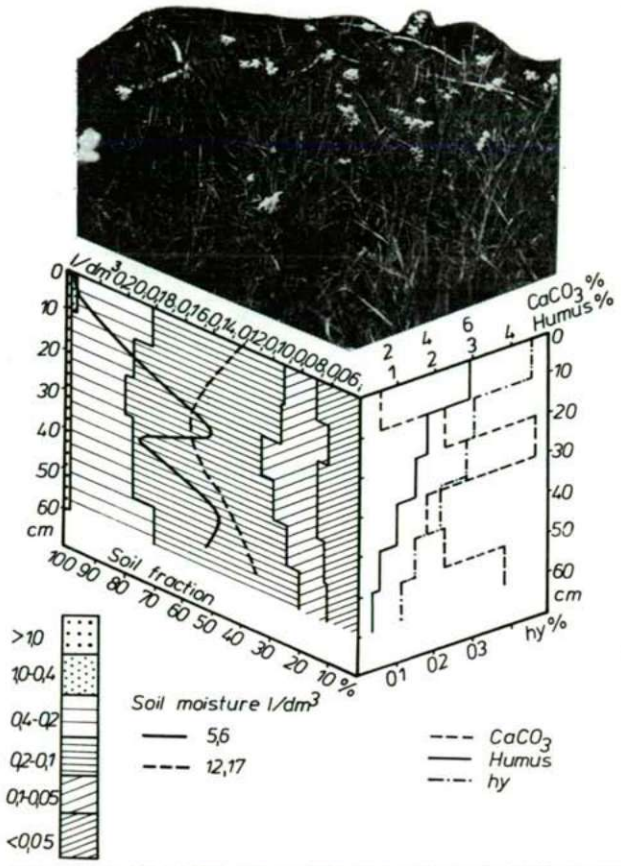

Fig. 10. Data on the soil profile of the *Filipendula* type zone among the sand-hills.

F(r)–S.r. molinietosum coeruleae (n.n)

It occurs in the deepest wind furrows in the area of sandy forest-steppe type of Emlékerdő, albeit its occurrence has also been sporadic a querter of a century ago. Independently from this, it forms good connections with *Molinio-Salicetum rosmarinifoliae* described from other areas of the Duna–Tisza midregion. Namely, these spaces among the hillocks must have been more deeper-lying in the past than today. In one of the wind furrows, the soil surface of the old marshy meadow is found at 80 cm depth. Its hy value indicates that the humus meadow sandy soil is harder here (BODROGKÖZY, 1957). Its present level was formed by the rearrangement of the sand masses in the region. Following the invasion and closing of *Festuca rupicola* in this area further superposition of sand could not have taken place. Later, the accumulation of organic matter on the surface resulted in the formation of humuscontaining sandy surface soil.

Hydroecology

The moisture content of its soil as expressed in lit dm^{-3} unit was never as high in the vernal aspect measured after a more abundant atmospheric precipitation as in the zone among the hillocks exposed to the shading effect of the trees. In late

summer the dynamics of water proved to be unfavourable in the soil of this subassociation, too (Figs. 12, 13).

Because of the competition for space for root caused by the closing of *Festuca rupicola,* its cenoses contain less species than the previous subassociations. Of the character species of the original *Molinio-Salicetum rosmarinifoliae* only *Serratula*

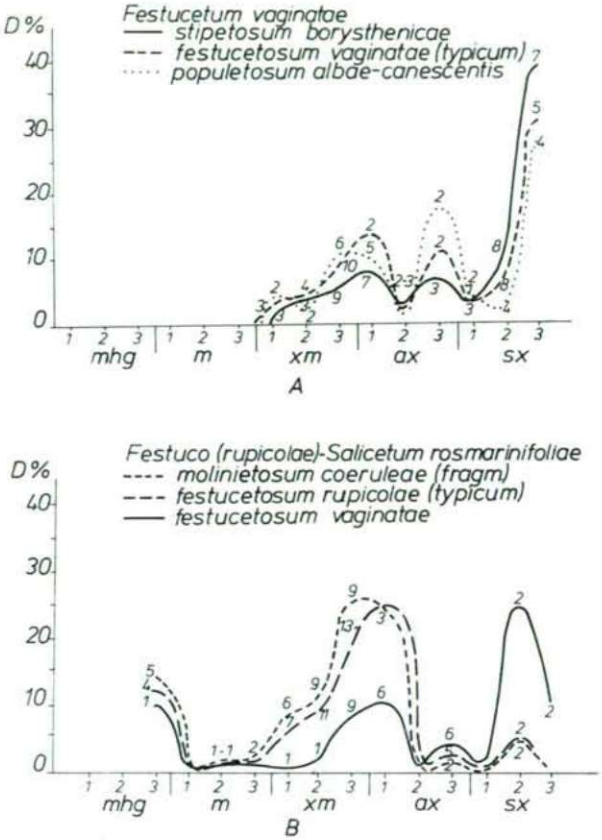

Fig. 11. Contributions to cover of plant communities on sand hill top (A) in the spaces between sand-hills (B) according to subgroups (1–3) of hydroecological categories and the number of species.

tinctoria, Thalictrum simplex v. *galioides* (both mhg 3) as elements of Molinion resp. Molinietalia can be recovered by stems. Their ecological amplitude was found to be extremely wide. These have namely survived in the stands of *Festuco-Salicetum rosmarinifoliae* festucetosum vaginatae of the nature conservation area, and in the last quarter of a century have not exhibited any noteworthy changes. On the other hand, *Linum catharticum* perished. At the same time, the Festucetalia valesiacae species, *Chrysopogon gryllus* as well as *Anthericum ramosum* the relict species of old steppe oak forests have immigrated. During the last decades, *Stipa capillata* has become facies-forming species and spread very greatly (Table 2).

Table 2. Festuco (rupicolae) -Salicetum rosmarinifoliae molinietosum coeruleae (1), festucetosum rupicolae (2) festucetosum vaginatae (3).

						Subass.:	1	2	3
	Steno-xerophyta:								
H	Festucion vaginatae	*Festuca vaginata*	sx3	F 3	T 4	N 1			
H	Festucion vaginatae	*Euphorbia seguieriana*	sx3	F 1–2	T 3	N 1			
H	Festucion vaginatae	*Stipa capillata*	sx2	F 2	T 3–4	N 2			
G	Festucetalia vaginatae	*Carex liparicarpos*	sx2	F 2	T 4	N 2			
H	Festucion vaginatae	*Carex arenaria* ssp. *tauscheri*	sx2	F 2	T 4	N 1			
H	Festucion vaginatae	*Onobrychis aranifera*	sx2	F 1	T 3	N 1			
H	Festucion vaginatae	*Syrenia cana*	sx2	F 2	T 4	N 1			
H	Festucion vaginatae	*Tragopogon floccosum*	sx2	F 1–2	T 4	N 1			
Th	Festucetalia valesiacae	*Bromus squarrosus*	sx2	F 2	T 4	N 2			
Th	Bromion tectorum	*Secale silvestris*	sx1	F 1–2	T 4	N 1			
H	Festucetalia valesiacae	*Anthericum ramosum*	sx1	F 2	T 4	N 2			
	Asteno-xerophyta:								
Th	Festuco-Brometea	*Verbascum lychnitis*	ax3	F 2	T 3	N 2–3			
Ch	Festucetalia valesiacae	*Thymus marschallianus*	ax3	F 1–2	T 3	N 1			
H	Festuco-Brometea	*Botriochloa ischaemum*	ax3	F 2	T 3–4	N 2			
H	Quercetea	*Inula salicina* v. *denticulata*	ax3	F 2–3	T 3	N 2			
Th	Festucetalia valesiacae	*Viola kitaibeliana*	ax3	F 2	T 4	N 2			
H	Festuco-Brometea	*Linaria genistifolia*	ax3	F 2	T 4	N 2			
H	Populetalia	*Calamagrostis epigeios*	ax3	F 2–3	T 3	N 3			
H	Festucetalia valesiacae	*Festuca rupicola*	ax1	F 2	T 3	N 2			
H	Festucetalia valesiacae	*Asperula cynanchica*	ax2	F 1–2	T 4	N 1			
H	Festuco-Brometea	*Teucrium chamaedrys*	ax1	F 1–2	T 2	N 2			
H	Festuco-Brometea	*Phleum phleoides*	ax1	F 2	T 3	N 2			
H	Festuco-Brometalia	*Poa bulbosa*	ax1	F 1–2	T 3–4	N 1			
G	Festuco-Brometea	*Muscari racemosum*	ax1	F 2	T 4	N 2			
Th	Festuco-Brometea	*Saxifraga tridactylites*	ax1	F 2	T 3	N 2			
H	Festucetalia valesiacae	*Campanula sibirica*	ax1	F 2	T 3–4	N 1–2			
	Xero-mesophya:								
H	Festuco-Brometea	*Poa angustifolia*	xm3	F 2	T 2	N 3			
H	Festuco-Brometea	*Euphorbia cyparissias*	xm3	F 1–2	T 0	N 0			
H	Festucetalia valesiacae	*Astragalus onobrychis*	xm3	F 1	T 3	N 1			
H	Festucetalia valesiacae	*Potentilla arenaria*	xm3	F 1–2	T 3	N 2			
H	Festucetalia valesiacae	*Stachys recta*	xm3	F 1–2	T 3	N 2			
Th	Festuco-Brometea	*Seseli annuum*	xm3	F 1–2	T 3	N 2			
Th	Festuco-Brometea	*Odontites lutea*	xm3	F 2	T 4	N 1–2			
H	Festucion rupicolae	*Astragalus austriacus*	xm3	F 1	T 3	N 1			
H	Festucetalia valesiacae	*Anthyllis vulneraria* ssp. *polyphylla*	xm3	F 1–2	T 3	N 1			
H	Festucetalia	*Cynanchum vincetoxicum*	xm3	F 1–2	T 3	N 2			
Th	Festuco-Brometea	*Erophyla verna*	xm3	F 2–3	T 0	N 1–2			
H	Festuco-Brometea	*Eryngium campestre*	xm3	F 1	T 4	N 2–3			
Th	Festuco-Brometea	*Arabis recta*	xm3	F 2	T 4	N 2			
Th	Festucion rupicolae	*Thesium arvense*	xm3	F 2	T 3	N 2			
Ch	Festucetalia	*Veronica prostrata*	xm3	F 2	T 3	N 1–2			
H	Festucetalia valesiacae	*Verbascum phoeniceum*	xm3	F 2–3	T 4	N 2			
H	Festuco-Brometea	*Salvia pratensis*	xm2	F 2	T 2	N 2			
H	Festucetalia valesiacae	*Dianthus pontederae*	xm3	F 2	T 4	N 2			
G	Festuco-Brometea	*Asparagus officinalis*	xm2	F 2	T 3	N 2–3			
H	Festuco-Brometea	*Coronilla varia*	xm2	F 1–2	T 3	N 1–2			

						Subass.:	1	2	3
G	Festucetalia valesiacae	*Iris humilis* ssp. *arenaria*	xm2	F 2	T 3	N 1–2	.....	.....	
H	Festuco-Brometea	*Medicago falcata*	xm2	F 1–2	T 3	N 2	.....	.....	
H	Festucion rupicolae	*Linum austriacum*	xm2	F 1–2	T 3–4	N 1	.....	.....	
H	Festuco-Brometea	*Ononis spinosa*	xm2	F 2–3	T 3	N 3		.....	
H	Festucetalia	*Scorzonera purpurea*	xm2	F 2	T 4	N 2	.....	.....	
Th	Festuco-Brometea	*Thlaspi perfoliatum*	xm2	F 3	T 3–4	N 2		.....	
Th	Festuco-Brometea	*Carduus nutans*	xm2	F 2–3	T 0	N 3–4		.....	
Th	Festuco-Brometea	*Erigeron acris*	xm2	F 2–3	T 2	N 2–3	.....		
M	Populetalia	*Populus alba*	xm1	F 0	T 3	N 1	———	.....	.....
H	Festuco-Brometea	*Galium verum*	xm1	F 0	T 2–3	N 1–2	———	■■■■■	
H	Festuco-Brometea	*Filipendula vulgaris*	xm1	F 3	T 3	N 1	———	■■■■■	
H	Festucetalia valesiacae	*Knautia arvensis* ssp. *arvensis*	xm1	F 2–3	T 2–3	N 0	.....	.....	
Th	Festuco-Brometea	*Holosteum umbellatum*	xm1	F 2	T 4	N 2–3	.....	.....	
Th	Secalietea	*Lithospermum arvense*	xm1	F 2	T 2	N 2–3	.....	.....	
Th	Chenopodietea	*Senecio vernalis*	xm1	F 2–3	T 3–4	N 3		.....	
		Mesophyta:							
Th	Arrhenatheretea	*Tragopogon orientalis*	m3	F 2–3	T 2–3	N 2–3	———	———	
Th	Secalietea	*Veronica arvensis*	m3	F 3	T 2	N 3		.....	.....
H	Molinio-Arrhenath.	*Lotus corniculatus*	m2	F 0	T 0	N 2–3	———	.....	
H	Molinio-Arrhenath.	*Ranunculus acris*	m2	F 0	T 0	N 2–3		———	
		Meso-hygrophyta:							
H	Molinietalia	*Salix rosmarinifolia*	mhg3	F 3	T 2	N 1–2	■■■■■	■■■■■	■■■■■
H	Molinio-Arrhenath.	*Polygala comosa*	mhg3	F 0	T 3	N 1	———	.....	
G	Festucion vaginatae	*Holoschoenus romanus*	mhg1	F 0	T 4	N 2	.....	.....	.....
H	Molinietalia	*Thalictrum simplex* v. *galioides*	mhg3	F 3–4	T 3–4	N 2	———	.....	
H	Molinio-Arrhenath.	*Serratula tinctoria*	mhg3	F 3–4	T 3	N 2	.....		
		Hygrophyton:							
H	Molinion	*Molinia coerulea*	hg 1	F 2–3	T 3	N 1–2	.....		

Festuco (Querco)-Populetum albae (Soó 71 nom. prov) n.n.

(Syn.: *Festuco (Populo) — Quercetum* (HARGITAI 40) Soó 71 n. prov. *Festuco-Quercetum* populetosum albae BODRK. 57).

Both for its species and its ecological conditions, this association is related to the stands of *Junipero-Populetum albae* (RAPCS. 22) ZÓLYOMI ex Soó 50 em. SZODTFRIDT 69. Occurrence and differentiation of its subunits of association seem to be connected with the places of occurrence of the ancient oak forests of sandy steppe type in our area. The *Populus alba* and *P. canescens* and their forms of transition (ex verb I. MARÓTI) could form well developed stands only in such places, where sufficient nutrient and moisture supply was secured for them by the humous sandy or brown forest soil covered by way of secondary sand movement in ancient oak forests (BODROGKÖZY, 1957). As a consequence of gradual drying in the course of historical times, the stands of *Populus* species do not proliferate any more generatively. They can only renew vegetatively. The process of ageing has been, however, so severe that no renewal could be observed even under the favourable light conditions of

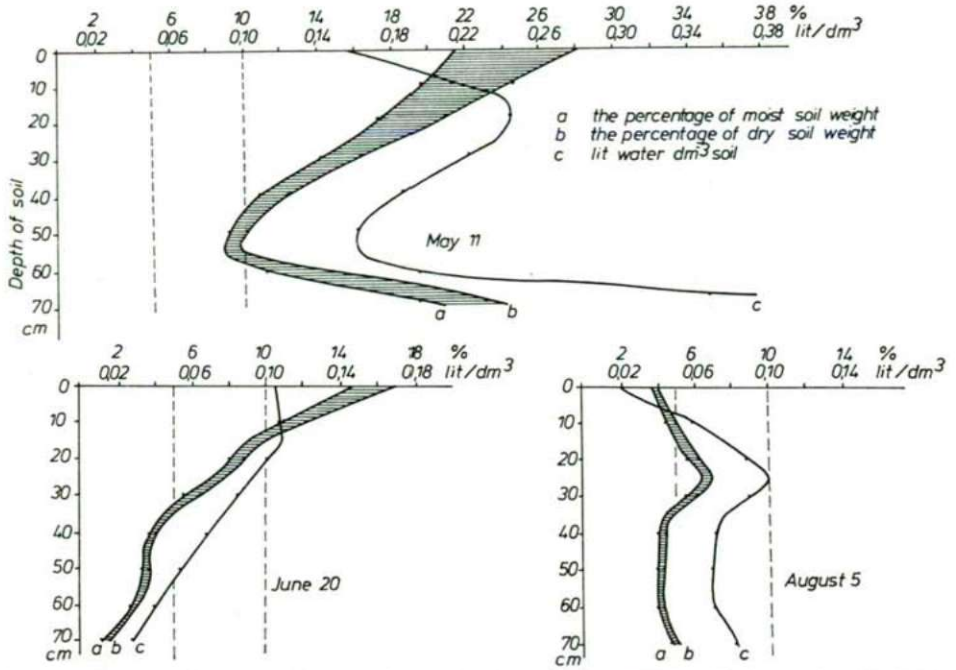

Fig. 12. Changes of soil humidity in the meadow between sand-hills with fragments of *Molinia* subass during the vegetation period.
a: the percentage of moist soil weight; b: the percentage of dry soil weight;
c: lit water dm³ soil.

gallery-forest-like stands. In our plain the frequently occurring violent wind-storms can be the cause of great havoc in the about 80-year-old poplar stands. Thus we have to reckon with the gradual extinction of these stands.

Ecological conditions

Its soil profile contains a very small silty fraction and the fine sand dominates (Fig. 14). The buried humus level occurs everywhere (BODROGKÖZY, 1957). On the basis of that and on that of the different degrees of water supply we can distinguish the following subunits of this association:

F.(Qu.)–P.a. calamagrostetosum BODRK. (57) n. corr.

(Syn.: *Festuco-Quercetum* populetosum albae xerophilum Bodrk. 57).

It is found on the sand ridges surrounding the depressions, where the buried humus layer contains less organic matter and occurs below 100 cm depth.

It is characteristic of its hydroecological condition that the ratio of moisture content per dry soil weight to that per wet soil weight and the ratio of moisture content in lit per dm³ dry soil to that of the wet soil was only in the vernal aspect, principally in the surface-near soil layers favourable in 1980. By the end of summer the value for moisture content was only 60 ml cm³. Changes in these values are illustrated in Figs. 14 and 15.

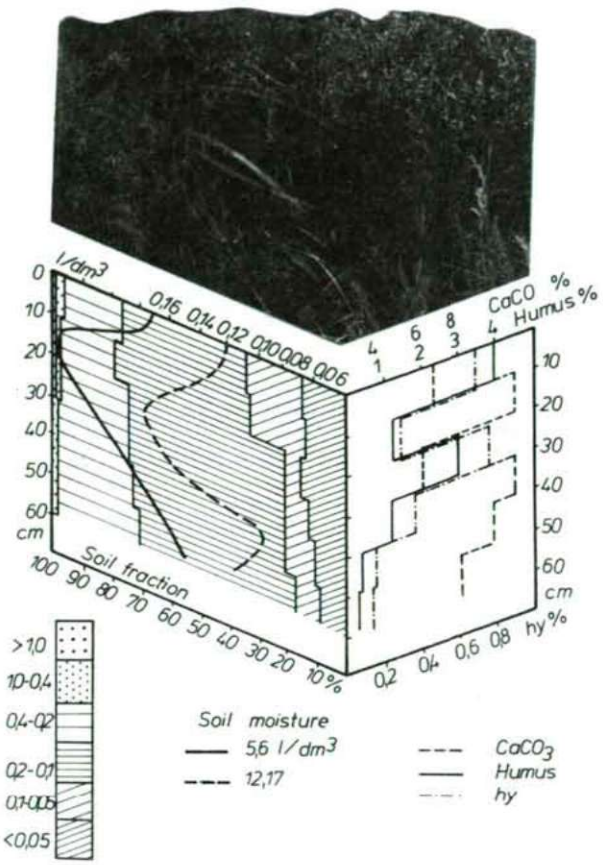

Fig. 13. Analytical data of the soil profile between sand-hills with fragments of *Molinio-Salicetum*.

Species composition

Its tree stratum is made up of *Populus alba* and *P. canescens* with never closing foliage. Its shrub stratum is made up of *Crataegus monogyna, Berberis vulgaris; Juniperus communis* is missing. Its herb stratum is fairly well lighted, which explains the dominance of species of Festucetalia valesiacae and Festuco-Brometea there and the increasing abundance of type ax3 *Calamagrostis epigeios*. This species has two well differenciable ecotypes in the Great Plain. It can be regarded as a meso-hygrophyte in its habitats along the rivers, and astenoxerophyte in the sandy steppes.

Among its species xeromesophytes also occur with greater species number besides steno- and astenoxerophytes. The xeromesophytes belong to the xm3 subtype. *Poa angustifolia* is a dominant species. The contributions of *Galium verum, Dianthus pontederae* etc. to coverage are smaller (Table 3, Fig. 16).

F. (Qu)–P.a. festucetosum rupicolae (typicum) n.n.

It occurs in more favourable situations than the previous one. Its tree stratum is more closed and the species composition of its herb stratum changes. Its shrub

Table 3. Festuco (Querceto) — Populetum
calamagrostetosum (1), festucetosum rupicolae (2), salicetosum rosmarinifoliae (3).

							Subass.:	1	2	3
MM	Populetalia	*Populus alba*	xm1							
MM	Populetalia	*Populus canescens*	xm1							
M	Quercetea	*Crataegus monogyna*	xm3	T 2–3	T 4	N 2				
M	Quercetea	*Berberis vulgaris*	xm3	F 2	T 4	N 2				
M	Quercion pubesc.	*Ligustrum vulgare*	xm2	F 2–3	T 3	N 2				
M	Molinietalia	*Salix repens* ssp. *rosmarinifolia*	mhg3	F 3	T 2	N 1–2				
M	Quercetea	*Cornus sanguinea*	xm2	F 2–3	T 4	N 2				
	Lawn-level									
		Steno-xerophyta:								
H	Festucetalia vag.	*Festuca vaginata*	sx3	F 2	T 4	N 1				
H	Festucetalia vag.	*Tragopogon floccosum*	sx3	F 1–2	T 4	N 1				
H	Festucion vag.	*Onobrychis aranifera*	sx2	F 1	T 3	N 1				
H	Festucetalia vag.	*Carex liparicarpos*	sx2	F 2	T 4	N 2				
		Asteno-xerophyta:								
H	Populetalia	*Calamagrostis epigeios*	ax3	F 1–2	T 3	N 3				
H	Festucetalia vales.	*Asperula cynanchica*	ax2	F 1–2	T 4	N 1				
H	Festucetalia vales.	*Festuca rupicola*	ax1	F 2	T 3	N 2				
H	Festucetalia vales.	*Festuca valesiaca*	ax1	F 2	T 3	N 2				
H	Quercetea	*Thalictrum minus*	ax1	F 1–3	T 2–3	N 1–2				
Ch	Festuco-Brometea	*Teucrium chamaedrys*	ax1	F 1–2	T 2	N 2				
H	Festucetalia vales.	*Viola rupestris* ssp. *arenaria*	ax1	F 2	T 2	N 1–2				
H	Festucetalia vales.	*Campanula sibirica*	ax1	F 2	T 3–4	N 1–2				
H	Quercetea pubescent.	*Senecio integrifolius*	ax1	F 2–3	T 3	N 2				
H	Quercetea pubescent.	*Hieracium bauhini*	ax1	F 2	T 3	N 2				
H	Festuco-Bromea	*Hypericum perforatum*	ax1	F 2–3	T 0	N 2–3				
H	Festucetalia vag.	*Silene otites* ssp. *pseudotites*	ax1	F 2	T 3	N 2				
Th	Chenopodietea	*Bromus sterilis*	ax1	F 2	T 3	N 4–5				
H	Festuco-Sedetalia	*Poa bulbosa*	ax1	F 1–2	T 3–4	N 1				
H	Festuco-Brometea	*Phleum phleoides*	ax1	F 2	T 3	N 2				
G	Quercetea	*Anthericum ramosum*	ax1	F 2	T 3	N 2				
		Xero-mesophyta:								
N	Quercetea pubesc.	*Cytisus austriacus*	xm3	F 1–2	T 4	N 1				
H	Festucetalia vales.	*Trifolium montanum*	xm3	F 2	T 2	N 1				
H	Festucetalia vales.	*Potentilla arenaria*	xm3	F 1–2	T 3	N 2				
H	Festucion rupicolae	*Astragalus austriacus*	xm3	F 1	T 3	N 1				
H	Festucion rupicolae	*Astragalus asper*	xm3	F 1–2	T 4	N 1				
H	Festucetalia vales.	*Astragalus onobrychis*	xm3	F 1	T 3	N 1				
Th	Festuco-Brometea	*Vicia angustifolia*	xm3	F 0	T 3–4	N 2–3				
TH	Secalietea	*Falcaria vulgaris*	xm3	F 3	T 0	N 0				
TH	Festuco-Brometea	*Seseli annuum*	xm3	F 1–2	T 3	N 2				
H	Festuco-Brometea	*Scabiosa ochroleuca*	xm3	F 1–2	T 3–4	N 1				
H	Festuco-Brometea	*Euphorbia cyparissias*	xm3	F 1–2	T 0	N 0				
H	Festucion rupicolae	*Vinca herbacea*	xm3	F 1–2	T 4	N 1				
H	Festucetalia val.	*Stachys recta*	xm3	F 1–2	T 3	N 2				
Ch	Festucetalia val.	*Veronica prostrata*	xm3	F 2	T 3	N 1–2				
H	Festucetalia val.	*Veronica austriaca* ssp. *austriaca*	xm3	F 2–3	T 3	N 1–2				
H	Festucetalia val.	*Dianthus pontederae*	xm3	F 2	T 4	N 2				
Th	Festuco-Brometea	*Odontites lutea*	xm3	F 2	T 4	N 1–2				
H	Festuco-Bromea	*Hieracium pilosella*	xm3	F 2–3	T 0	N 2				
TH	Festucion vag.	*Thesium arvense*	xm3	F 2	T 3	N 2				

							Subass.:	1	2	3
H	Festuco-Bromea	*Melandrium album*	xm3	F 2–3	T 3	N 2				
H	Festuco-Brometea	*Poa angustifolia*	xm3	F 2	T 2	N 3				
H	Festuco-Brometea	*Ononis spinosa*	xm2	F 2–3	T 3	N 3				
H	Festuco-Brometea	*Medicago falvata*	xm2	F 1–2	T 3	N 2				
TH	Festuco-Brometea	*Medicago lupulina*	xm2	F 2–4	T 3	N 2				
H	Festuco-Brometea	*Coronilla varia*	xm2	F 1–2	T 3	N 1–2				
H	Festuco-Brometea	*Pimpinella saxifraga*	xm2	F 0	T 0	N 2–3				
TH	Onopordion	*Cynoglossum officinale*	xm2	F 1–2	T 2	N 3				
H	Festuco-Brometea	*Salvia pratensis*	xm2	F 2	T 2	N 2				
Th	Festuco-Brometea	*Thlaspi perfoliatum*	xm2	F 3	T 3–4	N 2				
H	Festuco-Brometea	*Achillea millefolium* ssp. *collina*	xm2	F 2	T 4	N 2				
H	Festucetalia val.	*Achillea pannonica*	xm2	F 2	T 4	N 2				
H	Festucetalia val.	*Scorzonera purpurea*	xm2	F 2	T 4	N 2				
H	Quercetea pubesc.	*Leontodon hispidus*	xm2	F 0	T 2	N 2–3				
H	Festuco-Brometea	*Taraxacum laevigatum*	xm2	F 1–2	T 3	N 2				
G	Festuco-Brometea	*Muscari racemosum*	xm2	F 2	T 4	N 2				
G	Festuco-Brometea	*Asparagus officinalis*	xm2	F 2	T 3	N 2–3				
G	Festucetalia vag.	*Iris. humilis* v. *arenaria*	xm2	F 2	T 3	N 1–2				
H	Quercetea pubesc.	*Inula salicina* v. *denticulata*	xm2	F 2–3	T 3	N 2				
H	Festuco-Brometea	*Galium verum*	xm1	F 0	T 2–3	N 1–2				
H	Festucetalia val.	*Knautia arvenis*	xm1	F 2–3	T 2–3	N 0				
Th	Secalietea	*Viola arvensis*	xm1	F 2–3	T 0	N 2–3				
Th	Chenopodietea	*Senecio vernalis*	xm1	F 2–3	T 3–4	N 3				
H	Festuco-Puccinell.	*Scorzonera cana*	xm1	F 2–4	T 4	N 1–2				
H	Festuco-Bromea	*Silene vulgaris*	xm1	F 2–3	T 3	N 2				
G	Festucion rupic.	*Gagea pusilla*	xm1	F 2	T 4	N 2				
G	Festuco- Brometea	*Ornithogalum umbellatum*	xm1	F 2–3	T 4	N 3				
G	Querco-Fagea	*Epipactis athrorubens*	xm1	F 2–4	T 3	N 2				
H	Festuco-Bromea	*Dactylis glomerata*	xm1	F 0	T 2–3	N 0				
		Mesophyta:								
H	Molinio-Arrhenath.	*Trifolium pratense*	m3	F 0	T 0	N 2–3				
Th	Chenopodietea	*Senecio vulgaris*	m3	F 3	T 0	N 3–4				
TH	Arrhenatheretea	*Tragopogon orientalis*	m3	F 2–3	T 2–3	N 2–3				
H	Molinio-Arrhenath.	*Taraxacum officinalis*	m3	F 2–3	T 0	N 2–3				
H	Arrhenatheretea	*Ranunculus acris*	m2	F 0	T 0	N 2–3				
H	Molinio-Arrhenath.	*Trifolium repens*	m2	F 0	T 0	N 2–3				
H	Molinio-Arrhenath.	*Lotus corniculatus*	m2	F 0	T 0	N 2–3				
H	Quercetea	*Lithospermum officinale*	m2	F 2	T 2	N 3				
Th	Chenopodio-Scler.	*Bilderdykia convolvus*	m2	F 0	T 3	N 3				
G	Quercetea	*Cephalanthera rurra*	m2	F 3	T 3–4	N 2–3				
Ch	Festucetalia vales.	*Genista tinctoria* ssp. *elatior*	m1	F 0	T 3	N 1–2				
Th	Arction	*Anthriscus caucalis*	m1	F 3	T 3	N 3–4				
		Meso-hygrophyta:								
H	Querciom pubesc.	*Thalictrum simplex* ssp. *galioides*	mhg3	F 3–4	T 3–4	N 2				
H	Molinio-Arrhenath.	*Polygala comosa*	mhg3	F 0	T 3	N 1				
Ch	Populetalia	*Solanum dulcamara*	mhg3	F 4–5	T 3	N 3				
G	Quercetea pubesc.	*Carex flacca* ssp. *cuspidata*	mhg3	F 0	T 3	N 2				
H	Molinio-Arrhenath.	*Agrostis stolonifera* ssp. *gigantea*	mhg1	F 3	T 0	N 2–3				

stratum is also becoming richer. Besides *Crataegus monogyna* and *Berberis vulgaris, Ligustrum vulgare* and *Cornus sanguinea* which may be regarded as relict species of ancient oak forests occur here as well as in the west part of the region between the Danube and the Tisza (KOVÁCS-LÁNG and SZABÓ, 1971). In its herb stratum, apart from *Festuca rupicola, Asperula cynanchica* has spred particularly.

F. (Qu)–P.a. salicetosum rosmarinifoliae (n.s.ass)

(Syn.: *Festuco-Quercetum* populetosum albae mesophilum BODRK. 57).

It is found in those spaces among the sand-hills of Emlékerdő, where organic matter-rich humus occurs at about 80 cm depth below the superficial layer. This formed once the surface soil of the ancient oak forests (BODROGKÖZY, 1957). The most beautiful poplar forests of the nature conservation area occur there.

Hydroecological conditions

For the closing of the upper tree stratum, atmospheric precipitation is stored mainly in the near-surface layers, remaining there throughout the whole summer season. Detailed data are presented in Fig. 17.

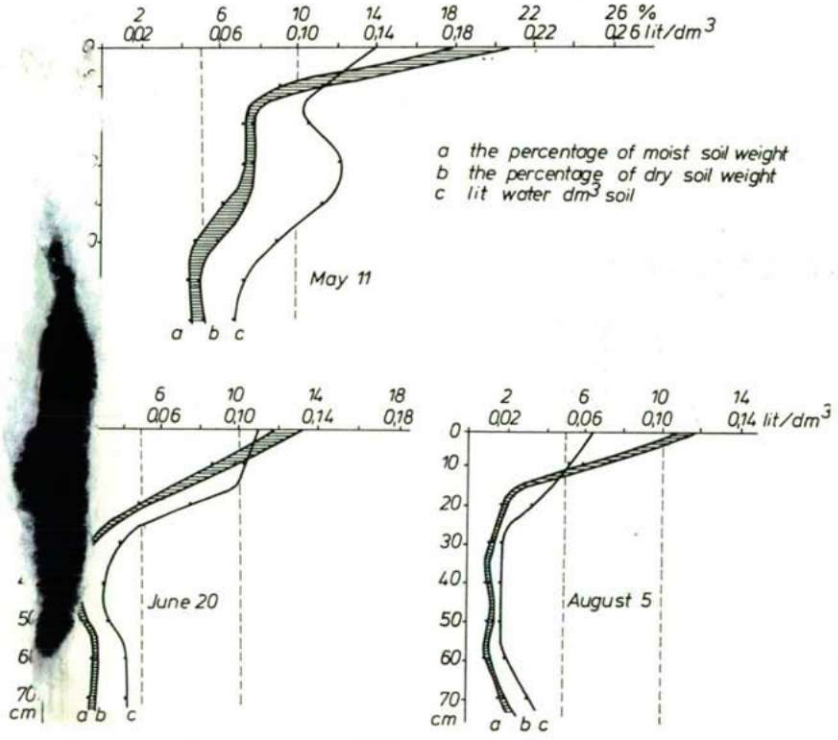

Fig. 14. Moisture dynamics in the soil profile of white poplar gallery forest of *Calamagrostis* type.

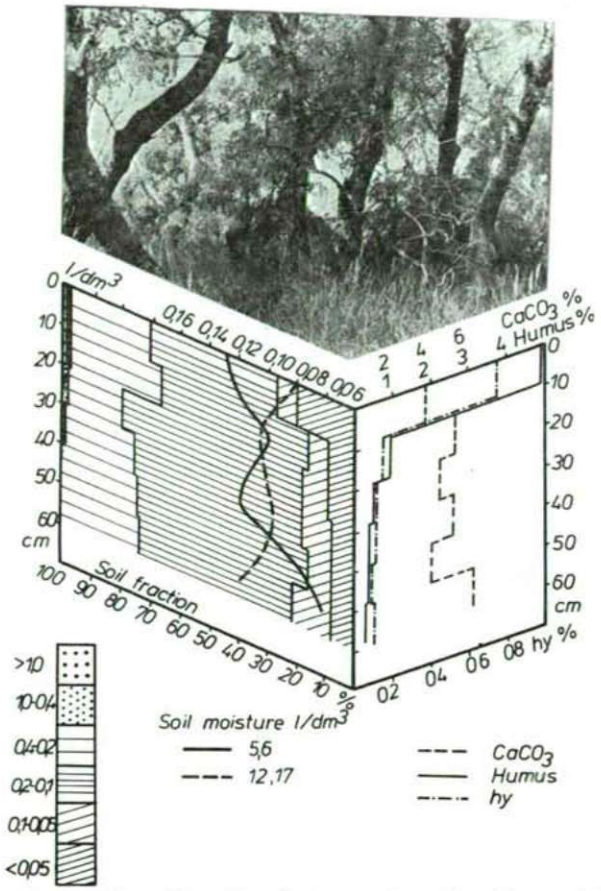

Fig. 15. Analytical data on the soil profile of whte poplar gallery forest of *Calama[illegible]ostis* type.

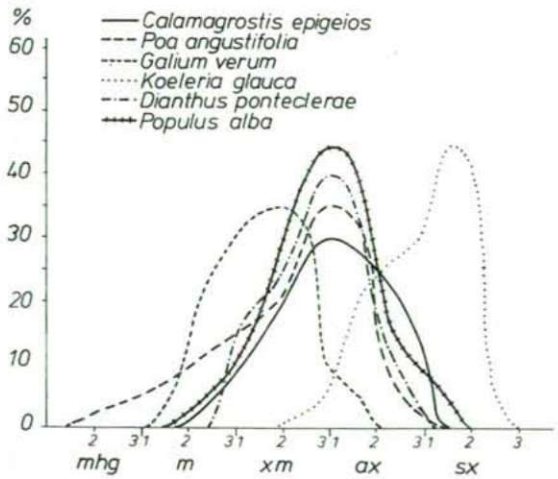

Fig. 16. Hydroecological graphs for the character species of *Festuco (Querco)* — ***Populetum albae*** calamagrostetosum.

Cenotical conditions

Changes occurred in the shrub stratum with the growth of *Salix repens* ssp. *rosmarinifolia*. Otherwise, it is like the stands of the type in respect of the number of species and the contribution to cover. The steno-xerophytes do not occur any more in its herb stratum, and the members of astenoxerophytes have also diminished. In the last decades, ax3 *Calamagrostis epigeios* has also increased to the expense of *Festuca rupicola*. The differential species of this subassociation are the *Campanula sibirica, Teucrium chamaedrys* (both ax1).

Members of xeromesophytes dominate in respect of species number, particularly the type xm3 and xm2 species. Nevertheless, their contributions to cover is little, with the exception of *Poa angustifolia*. Further details are presented in Table 3.

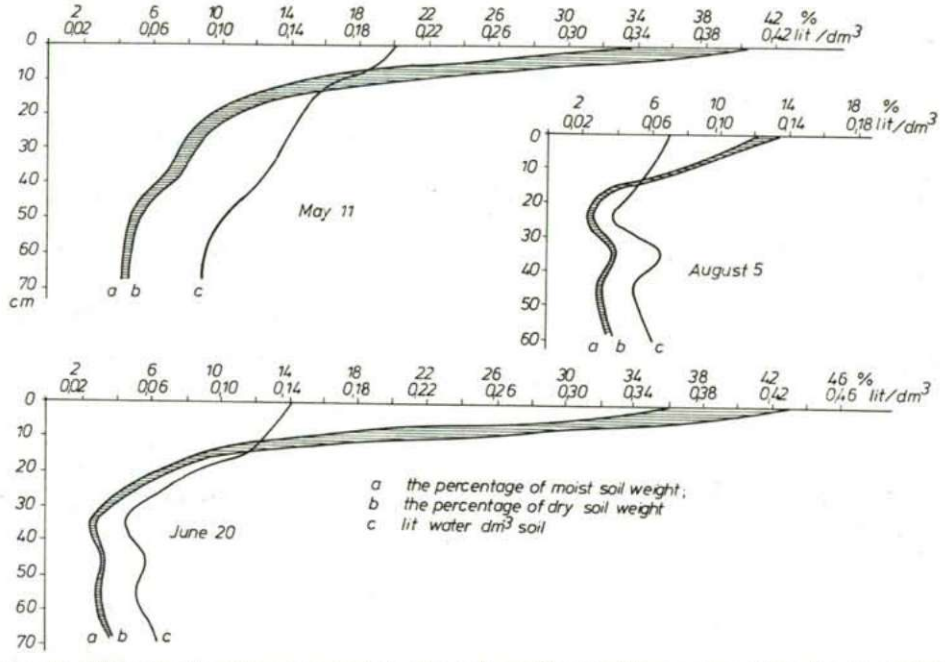

Fig. 17. Changes of moisture content in the soil profile of white poplar gallery forest of *Salix rosmarinifolia* type in the vegetation period, 1980.

Species occurring in forests and skirts of forests as *Thalictrum minus, Senecio integrifolius, Anthericum ramosum, Cytisus austriacus, Carex flacca* ssp. *cuspidata, Leontodon hispidus, Inula salicina* v. *denticulata Epipactis atrorubens, Cephalanthera rubra, Lithospermum officinale, Thalictrum simplex* v. *galioides* can be regarded as relict species of ancient oak forests, which is very significant from the aspect of environmental protection. The hydroecological plots for some of these species are presented in Figs 17 and 18.

The total contributions of species belonging to the single categories and subgroups of these categories to cover as well as the number of these species are seen in Figs. 19 and 20. The differences among the three subassociations are also apparent from these graphs.

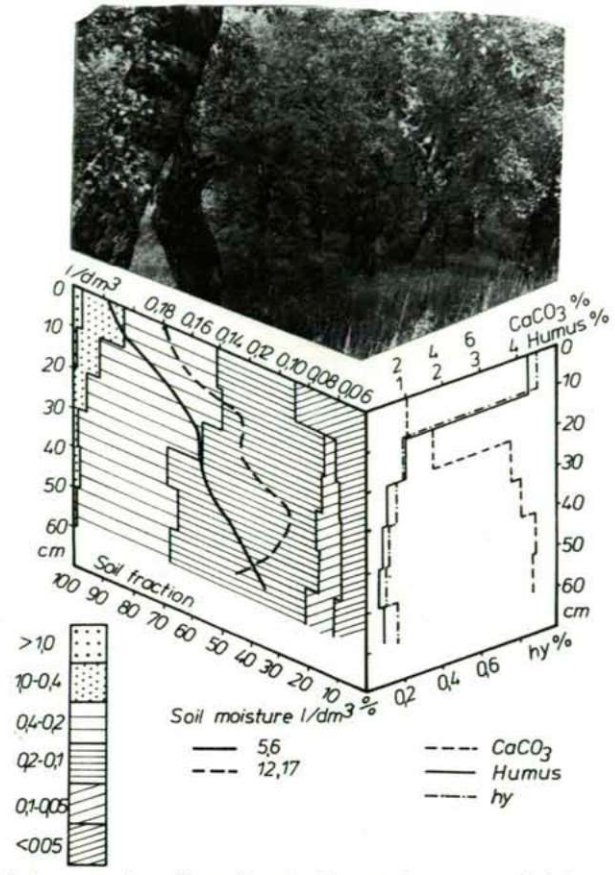

Fig. 18. Analytical data on the soil profile of white poplar grove of *Salix rosmarinifolia* type among sand hills in 1980.

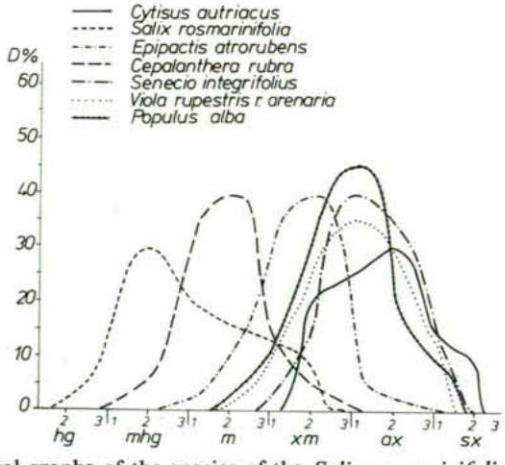

Fig. 19. Hydroecological graphs of the species of the *Salix rosmarinifolia* subassociation.

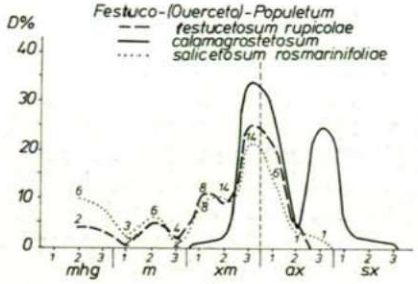

Fig. 20. Distribution of species of the single subunits of association according to their contributions to coverage, with special regard to the subgroups of categories (1–3) with the indication of species number.

Changes in phytomass production

The effect of ecological conditions on the phytocenoses is also evidenced by the seasonal changes of organic matter production. PRÉCSÉNYI (1967) and ZÓLYOMI and PRÉCSÉNYI (1970) dealt with the methodology of these studies. — Investigations of this kind in connection with the lichen cenology of sandy forest-steppe plant communities were performed by VERSEGHY and KOVÁCS-LÁNG (1974): Studies on grass cenoses were reported by KOVÁCS-LÁNG and SZABÓ (1971) as well as SIMON and BATANOUNY (1971), SIMON and KOVÁCS-LÁNG (1972).

PRÉCSÉNYI and OPAUSZKI (1979) investigated the concentrations of micro- and ultraelements, and HORÁNSZKY et al. (1980) performed morphological studies on the populations of *Festuca vaginata.*

Table 4. Changes in organic matter production of sand steppe plant communities in the vernal and aestival spects (1 = May, 2 = June).

		Grasses and sedges	Dicotyledones	Living matter	Dead matter	Sub-surface total production
		Total (g/m²)				
Festucetum vaginatae stipetosum bor.	1	38.64	26.60	65.24	253.32	318.56
	2	55.20	25.88	81.08	250.00	331.08
Festuco rupicolae salicetosum rosm.	1	42.96	101.32	144.28	482.64	626.92
	2	113.52	153.14	266.66	258.92	525.58
Fest. rup. Filipendula fac.	1	73.32	46.64	119.96	405.32	525.28
	2	149.16	163.00	312.16	206.10	518.26
Fest. rup. molinietosum Stipa capillata fac.	1	82.64	22.60	105.24	512.00	617.24
	2	194.40	82.20	276.60	299.60	576.20
Festuco (Querco) — Populetum calamagrostetosum	1	59.96	10.64	70.60	320.00	390.60
	2	251.20	62.56	313.76	111.40	425.16
Fest. (Qu.)-Pop. salicetosum rosm.	1	116.00	22.00	138.00	262.00	400.00
	2	194.40	82.00	276.40	299.60	576.00

Further supplementary studies were also performed in connection with the phytomass production of the aestival aspect of plant communities in the sandy forest-steppe of Emlékerdő at Ásotthalom. Their comparative data are presented in Table 4.

***

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PHYLOGENETIC IMPORTANCE OF THE XYLOTOMY AND GEOGRAPHICAL DISTRIBUTION OF HOMOXYLIC DRIMYS WINTERI AND DRIMYS COLORATA (2)

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Abstract

In the Introduction, the author gives a short survey of the xylotomic investigations into the homoxylic trees. He deals in details with pitting of the tracheids of homoxylic trees, more exactly of *Drimys winteri* and *Drimys colorata,* with the origin of the spiral and stepped thickening, with the phylogenetic importance of monoecism, dioecism, and of the hermaphroditic (bisexual) states. In ultimate analysis, he arrives at the conclusion that Drimyses and their relations (Tetracentron, Trochodendron), as anciant types, cannot have originated — on the basis of their xylotomic, phytogeographic and genetic particularity — from Magnoliales (sensu TAKHTADJAN). Probable, they — as vesselless plants of aggregated pith ray, of various sex, with amenta, tracheids — are genetically rather closer to the vesselles homoxylic Conifers and Amentiflorae of varying sex with amenta than to Magnoliales (sensu TAKHTADJAN) of more developed vessels and hermaphroditic flowers, functioning with a torus.

Introduction

The first phylogenetic monograph of the author „Ein Gedanke zur polyphyletischen Entwicklung der Pflanzenwelt", was published during World War I, in 1918 (cf.: References). Here he first proclaimed the doctrine of phylogeny of land plants in three directions, from the state of protophytes till the most developed plants, i.e. the angiosperms, including the origin of monocotyledons and dicotyledons, as well. Later on, an animated discussion was carried on in this field, particularly in connection with the homoxylic trees. Concerning this, in Metcalfe's book the following are to be read:

Bailey and Nast in their study of the morphology of this group have shown that, with the exclusion of *Illicium*, the Winteraceae become a homogeneous, natural aggregation of obviously closely related plants. Within the group, these authors distinguish the following trends of structural specialization: "toward reduction or elimination of wood parenchyma in Sect. *Wintera* of *Drimys*, toward excessively widened multiseeiate rays in *Pseudowintera*, and toward reduction of cell size, particularly in dwarfed or microphyllous species e.g. in Sect. Tasmannia of *Drimys*."

The occasional scaliform pitting that occurs in the tracheids of *Drimys*, figured largely in the controversy of about 1918, assiciated with the names of BAILEY and JEFFREY, as to whether *Drimys, Tetracentron,* and *Trochodendron* represent degenerate evascularized Dicotyledons, the ancestors of which possessed true vessels in their secondary xylem, or whether they have descended directly from ancestors that possessed scalariform tracheids in their secondary xylem. Modern work on the significance of the length of the cambial initial and vessel members has provided a means of testing such hypotheses and BAILEY and NAST, in 1945 stated that the primitive character of the cambium and xylem in the Winteraceae, *Trochodendron* and *Tetracentron* rules out any possibilities of these plants having developed vessels and subsequently having lost them. Elsewhere they claim that, when evidences

from all organs and parts of the plants are taken into consideration, there are no convincing arguments for deriving the Trochodencraceae from the Winteraceae or vica versa, or even for inferring that these families are closely related genetically. Nor con one assume that the other ranalian families were derived from these vesselless and the Coniferae overlook important anatomical differences, such as occur in the rays, and are thus misleading. They conclude that if the vesselless wood of the Winteraceae is to be compared with that of the Gymnosperms, it should be with the secondary xylem of Pteridospermae and Bennettitales rather than with that of the Coniferae, Ginkgoales or Cordaitales."

TAKHTADJAN's booklet of the origin of Angiosperms was published in Russian 1954, which he sent to the author of this paper as well. This book is of 96 pages extent, containing 16 illustrations. The 16th of these is showing a phylogenetic table, tracing the origin of angiosperms back to a single common stock, the Magnoliales. With this, he considers the origin of angiosperms as monophyletic. This theory is diamatrically opposed to the above theory of the author. In the meantime, other authors have also dealt with this problem, particularly with the peculiar structure and taxonomic position of homoxylic trees (ZIMMERMANN, LAM, ASAMA, etc.). Some of them consider particularly the homoxylic trees as degenerate forms: others are, however, adherents of the polyphyletic conception.

In 1954, the author presented his theory in Paris, in the International Botanical Congress, as well. In 1962, he dealt with this problem, again, making use of the recent literary data. The title of this monograph was: "The phylogeny of sexuality and triphyletic evolution of land plants". In this work, he repeats his older opinion, regarding the origin of land plants.

TAKHTADJAN's work, entitled "Evolution und Ausbreitung der Blütenpflanzen" was published in 1975. His phylogenetic theory has been published here repeatedly. In the mean time, his theory was reviewed, and partly even accepted, in more than one country. Thus, among others, the Hungarian professor REZSŐ SOÓ has entirely taken over TAKHTADJAN's system in his university textbook (Phylogenetical phytotaxonomy). And even, in the University Botanical Gardens in Szeged, ENGLER's system was transformed and replaced by TAKHTADJAN's system.

In the meantime, however, this system has been criticized by several authors. Thus, among others, by Gottwald in Hamburg. He proves on a very wide xylotomic basis that Magnoliales cannot be the ancient angiospermic type because there are some plant families and orders, too, carrying much younger characters. In his paper, he writes: "Abstract". The stem wood of about 700 species, belonging to 32 families of the order Magnoliales s.l., plus further taxa exhibiting primitive wood anatomical features were investigated. On this basis, six structural groups can be established each of which shows a marked gradation from primitive to advanced stages. Wood structure of Magnoliales sensu TAKHTADJAN is only partially primitive, partially moderately derived, while the most primitively structured heteroxylous taxa belong to the "Dillenial-Hamamelidal" and "Theal" groups, respectively. Accordingly, there is no compelling evidence to support phylogenetic schemes in which the Magnoliales is placed as the only common base for all recent dicotyledons."

The authors has got the opportunity in 1979, to procure some wood of homoxylic *Drimys* winteri and to investigate into it from xylotomic point of view. He renders account of the results of his investigations in the following way.

"The author considers, contrary to TAKHTADJAN's theory of monophyletic origin, that a polyphyletic origin of the angiosperms is more probable and he wants

to support his opinion by xylotomy of the homoxylous *Drimys*, comparing it with a *Juniperus* and a *Magnolia*. As *Drimys* has only tracheids with simple pits and is without vessels, while in the Magnoliales vessels occur, and as the tracheic state is mor primitive than the vessel-containing one, the homoxylous trees could, therefore, not originate from Magnoliales".

Materials and Elaboration

In the following, we attempt to verify that Drimyses *(Drimys winteri, Drimys colorata)* cannot originate from the order Magnoliales, as supposed by the Soviet TAKHTADJAN and his followers, e.g. the Hungarian Soó. We will support our statements with photographs and arguments.

We will prove, why the origin of angiosperms cannot be monophyletic but only polyphyletic, why Magnoliales cannot be the prototype, from which all the mono- and dicotyledons, the monoecious and dioecious plants would have come (Plate I).

In Plate I, there are six photographs. They are showing the species of: 1) a gymnospermous tree *(Amentotaxus argotaenia)*, 2) and 4) the homoxylous *Drimys winteri*, 3) *Drimys colorata*, 5) *Alnus incana* (Amentiflorae), 6) Magnolia accuminata (Dialipetalae). The cross-section structures of the gymnospermous pine and of the two Drimyses (2 and 4) are very similar to one another. Vessels are missing in both. The whole stock consists of tracheids. Fig. 1. is showing the cross-section structure of *Amentotaxus argotaenia*. In the one cell-layer, elements of two sizes are arranged, between wide pith rays. The elements generally follow one another in radial direction. Between them, in a smaller or larger distance, there were generally arranged larger tracheids of regular cylinder-form and then smaller ones of angular cross-section. Tracheids of two different sizes seem to have developed already here, in pines; and from the larger ones may have been formed the later real tracheids and vessels, and from the smaller ones the parenchyma cells.

It is interesting that inside the wider tracheids spiral thicknesses lie. These vessels are, however, missing from the gymnospermous pine, the Drimyses. In this regard, Drimyses are closer to gymnosperms than to Magnoliales. It is interesting, too, that in both Drimyses aggregated pith rays also occur but these are missing from pines and Magnoliae.

In Figs. 2 and 4, there are two kinds of cell of cell types near to each other. In Fig. 4, this can be established well. The capital letters show the tracheids, while the cyphers rather the parenchymatic cells, radially somewhat elongated. In Fig. 5, about Amentiflorae *Alnus glutinosa*, the part of a pith ray is to be seen, where the tracheid series alternate with the mono-layer parenchyma. A fully identical structure is visible in the homoxylic *Drimys winteri*, as well (Fig. 4). On the other hand in Fig. 6, in the *Magnolia*, and also in its other relations, there do not occur any aggregated pith rays.

On the basis of the presence or the lack of the aggregated pith rays, Drimyses can rather be brought into a nearer genetic connection with Amentiflorae than with the Magnolis, belonging into the the Dialeptale. As such an ancient feature is entirely missing from Magnoliales, but they are to be found in Drimyses and Amentiflorae, they may therefore not have origined from Magnoliales, as imagined by TAKHTADJAN and his followers. For proving that this is an ancient feature and that it, as such one, has nevertheless remained, we enumerate here a number of such Amentiflora species: *Casuarina aequisetifolia, Alnus incana,* (Fig. 5) *Alnus glutinosa, Corylus avellana, Fagus sylvatica, Fagus orientalis, Quercus cerris, Quercus ilex, Quercus petrea, Quercus robur, Quercus borealis maxima, Carpinus betulus,* and even Ephedrae, etc., etc. Such aggregated pith rays cannot be found at all among the monocotyledonous trees — these having otherwise no pith rays — and occur among the dicotyledons, too, only in the rarest cases. It is proved — therefore, already on the basis of cross-sections that the development of angiosperms did not set out from Magnoliales. Before these were already the primitive homoxylic trees, the structure of which already reveals more details.

Pitting of the wood of Drimys winteri and Drimys colorata

The xylem of Drimyses is formed — as seen before — by large and only by tracheids and scanty parenchyma cells. A characteristic property of tracheids is that they are unicellular, in their walls only simple pits line up. In Fig. 7, the simple

Plate I

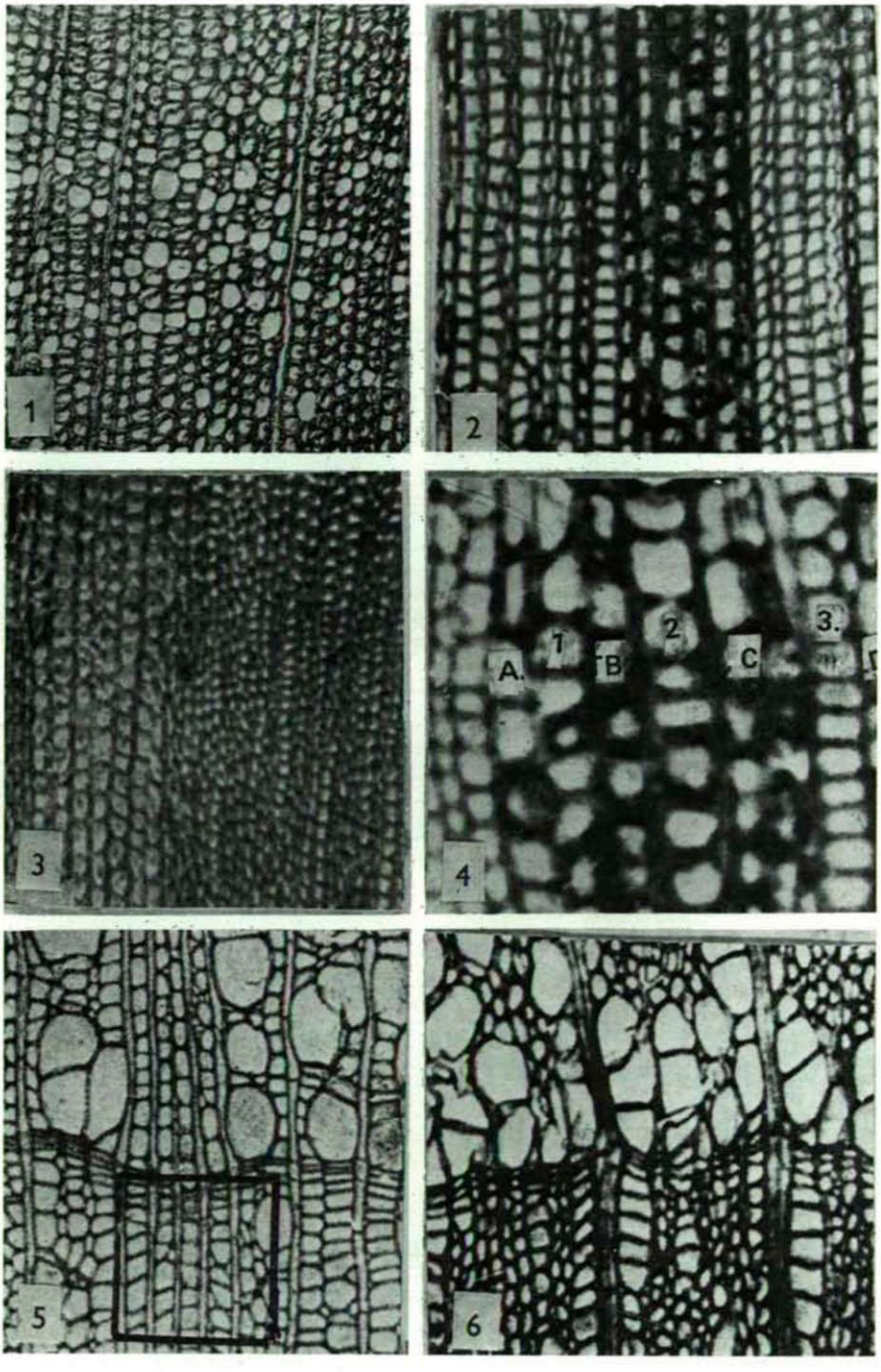
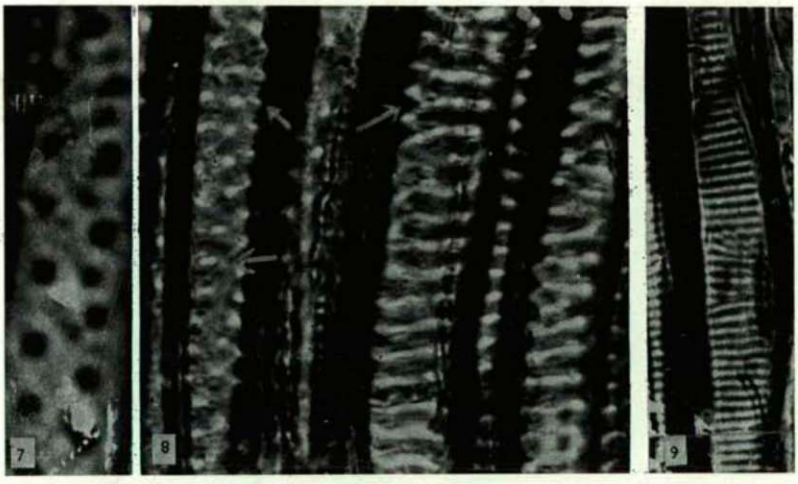


Plate I

1. Cross-sectional picture from the trunk of the gymnospermous pine *Amentotaxus argotaenia.* The cross sections of the two kinds of elements are separated well from each other. The major ones are tracheids, the minor ones are parenchyma cells, arranged in radial lines. The pith rays are singlelayer. This structure is rather similar to the *Drimys* than to Magnoliales (x100).
2. Cross-sectional structure of the homoxylic *Drimys winteri.* In the middle is running, separated, an aggregated pith ray. The matter of the xylem also consists of tracheids. Their cross-sectional structure is generally square, here as well. In their size they differ a little from the somewhat smaller rows, which probably are unicellular pith rays or parenchyma rows. This structure is more similar to that of Amentotaxus than to that of Magnoliales (x100).
3. Cross-sectional structure of *Drimys colorata.* On the left an aggregated pith ray is lying, separated by its major cells. The matter of xylem consists, here too, of tracheids. Vessels are entirely missing (x80).
4. Magnification of a detail of picture 2. The capital letters indicate the radially elongated parenchyma rows, while by ciphers the tracheid rows between these are indicated (x180).
5. Cross-sectional structure of the dicotyledonous Amentiflorae *Alnus incana.* In the ground tissue of the tracheid, between the parenchyma cells and the cornered tracheids, solitary twin poles and pore rays take place. In the black square, there is a detail of an aggregated pith ray (x200).
6. Cross-sectional structure of *Magnolia accuminata,* ranged into Dialipetaleae. In the xylem tracheids, single vessels, pore rays, and pith-ray cells take place. There are no aggregated pith rays. (x200).

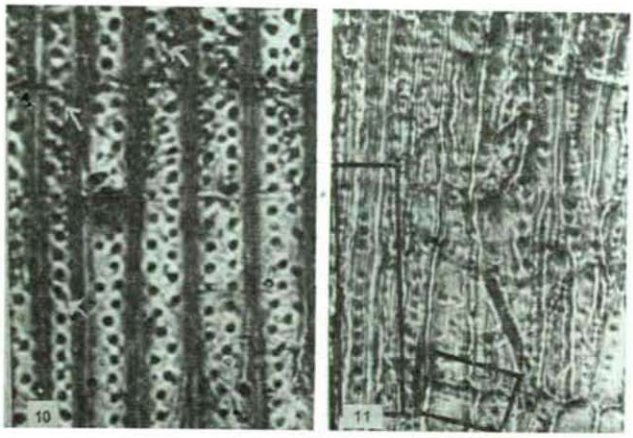
Plate I



7. Tracheid structure from *Drimys winteri.* In the wall of tracheids, the simple pits are arranged separatly and in alternating lines. There are no bordered pits (x150).
8. In the walls of eraly tracheids, the simple pits take place in pairs or in threes, spirally and in slanting rows. The pits are open or compact (see, on the left, at the white arrow). In the middle, the openings of pits take place spirally. These are not step-ladders, like those to be seen in Magnoliales. There is an essential difference between the two. Stepped thickness occurs only in Magnoliales. In Drimyses it doesn't (x150). occure.
9. Stepped thickness in Magnolia accuminata. In Drimyses, the edges of spirals are uneven, while in Magnoliae the steps of the stepped thickness are parallel and straight (x125).

pits are wellvisible, following one another alternatively. Another property of pittings is "oppositions", when the pits follows one another in horizontal or longitudinal lines. In Drimyses, no pits like this occur. It is interesting that in the walls of tracheids

Plate I



10. Six tracheids from the ray-side of *Drimys winteri*. In the walls of tracheids, the simple pits are arranged in 1 to 2 lines. (x175).
11. Radial side in the tracheids of *Drimys colorata*. In the framed rectangles there are thin-walled parenchyma cells, with single-line large, simple pits in their walls. (x160).

the simple pits are always located in a certain definite distance from each other. At the first glance, these regular circular openings are surrounded by light boundles and they look to us as if the micelles (?), forming the part of the wall, had been arranged in a sequence. In Figs 10 and 11 can be observed well enough.

Observing this, it seems to us as if the central pi were always be surrounded by two spiral plexuses, each. In Fig. 16 to the left from the cipher, we see such details. The two bundles, running in opposite directions, lie one above the other with lateral pits in them. Consequently, the tracheids form a tube-like system. The tube-like structures are to be seen very well in Fig. 16 in the part at the small cipher 2.

The situation will be still more complicated, when in the wall of the tracheids not only one but even two or three pits can take place, in an alternating position with the foveolae below them. All these are visible on the left of Fig. 8, where below one, and above it, two or three pits lie close together and apparently a ladder-like thickening comes into being. This is, however, no right observation because the simple pits being in an alternating situation, touching with one another, seem to be

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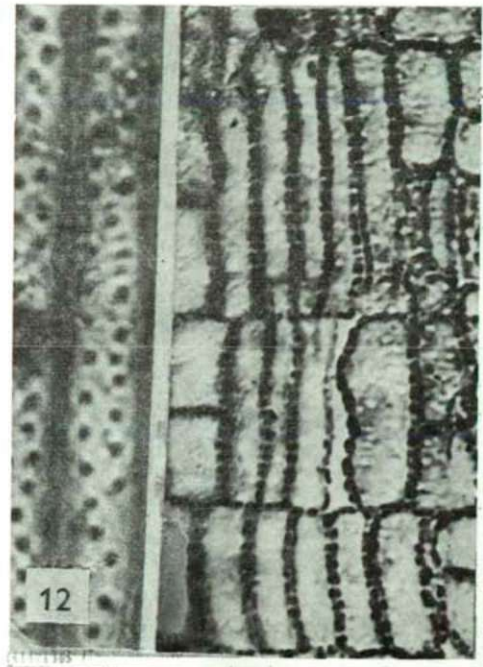
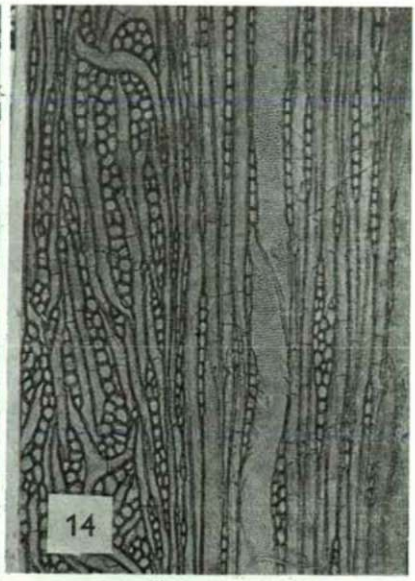
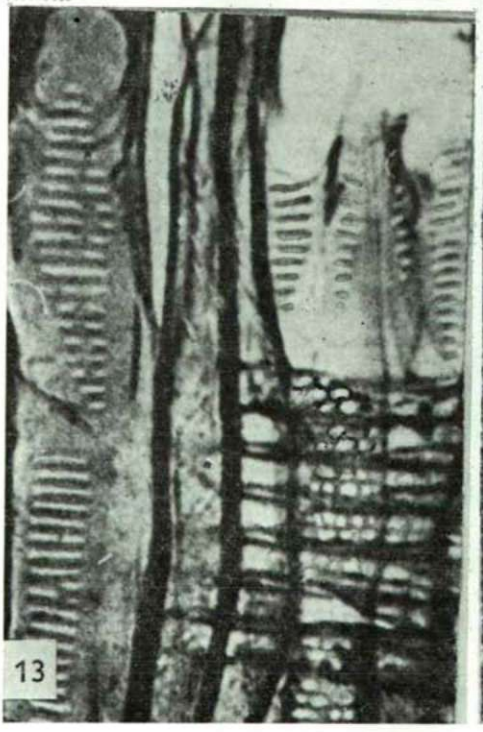
Plate I

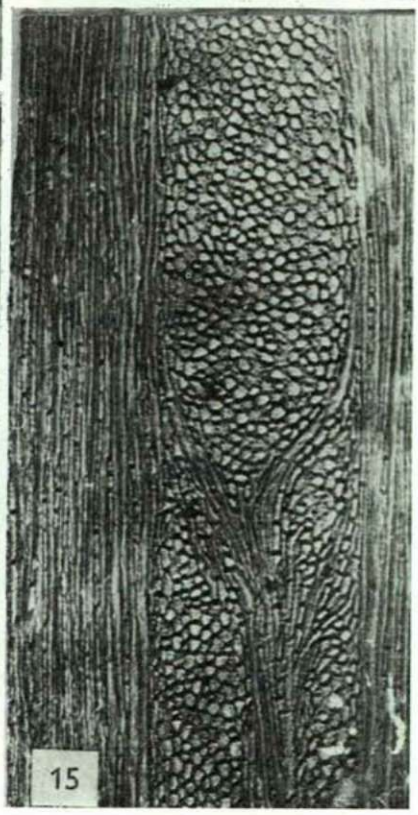
12. On the left, there are two elongated tracheids with simple pits. The same pitting is shown by the cells of the wide pith rays. On the right of the picture, a tracheid formulation is to be seen. The pith-ray cells are initially cube-like, divided vertically, becoming 4-cellular and, at last, longitudinal tracheids (x120).
13. Radial structure from *Magnolia accuminata*. On the left, there is a stepped vessel, above a simple perforation. On the right below, they are horizontally elongated. In the walls, touching the vessels, there are simple, round pits. This is also a difference between two kinds of pith rays (x120).
14. An aggregated pith ray from Amentiflorae *Alnus incana*, composed by more than one, one- or two-layer, smaller pith rays (x110).
15. An aggregated pith ray from *Drimys colorata*. (Tangential side). Tracheid basic substance and pith-ray rows (x110).

Plate I





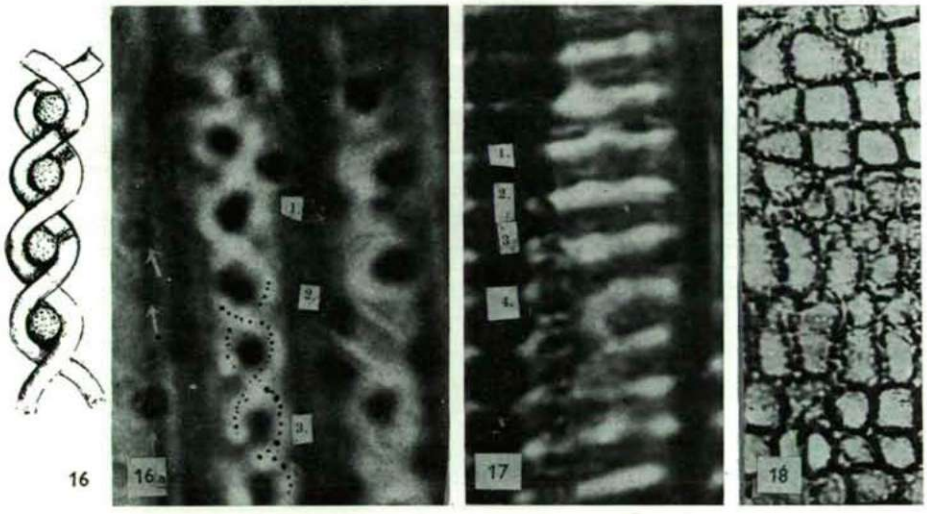




distinguishable counter-laths. For a perfunctory observation this seems to be a ladder-like thickening, whereas these are, in fact, spiral formations. The most interesting in these formations is that they are of tubular structure, they seem, therefore, to have inside small cavities. In Fig. 17 at the tiny ciphers, the tubules are torn, showing the tubular structure clearly.

This spiral structure essentially differs from the ladder-like thickening of Magnoliales (Fig. 9). Drimyses cannot have originated from Magnoliales, if only on the ground that the spiral structure is formed in another way than the ladder-like thickening. The explanation of that is in tracheids, these horizontal tubules seem to be step-ladders, the spiral lines are running in these paces cery close to one another. This is a tubular structure, something which could not be observed, so far, either in pines or in deciduous trees. It is proved by different anatomical structures, as well, that Drimyses cannot have originated from Magnoliales because their wood anantomies are essentially different from each other. (Cf. GOTTWALD.) That these spirals are tubules, it is proved by the circumstance, too, that in certain places tubules seem to be obstructed. This can be seen very well at the lower white arrow of Fig. 16, resp. to the left from cipher 16, at the edge. The ruptures can be observed well at the small ciphers 1, 2, 3, 4, 5, 6 of Fig. 17. At cipher 2, the cavities are to be seen at the ends of the two half-rings. Both ends of the crescent-like piece, No. 2. are torn, reminding us of a cross-cut ring.

Plate I



16. Simple pits in the walls of tracheids. On the left, at the arrow, there are simple pit-openings. At cipher 16 (in the corner), the cavity, thin wall of the tracheid is to be seen well. The dotted line (in the middle) is indicating the formation of two contrary bundles. At the small cipher 1, it is visible well enough, as the two bundles interwined surround, resp. form the simple pit. At the small cipher 2, the cavity and wall of the tubule is well-observable (x190).

16a. Formation of simple pits in the walls of the tracheid of Drimys winteri.

17. At the small sciphers, the tube-like openings of the cross-thicknesses are to be seen, indicating that these Thicknesses are of tubular and spiral structure (x190).

18. In the wide aggregated pith rays, the cube-shaped parenchyma cells are simply pitted, like those of tracheids (x75).

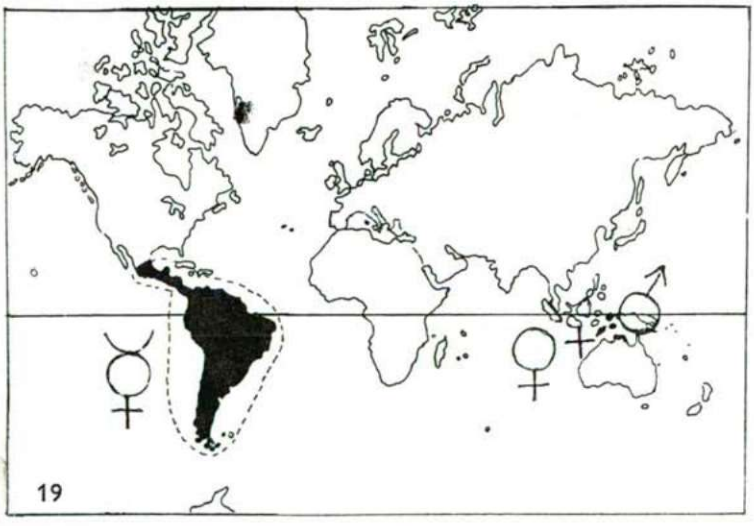
Another matter surprising fact is also shown by the structure of pith rays. Fig. 8, as mentioned, in Drimyses, some pith rays develop, as well; generally, they are one cell layer or 15–20 cells wide, and generally of heterogenious nature. In Fig. 12, the marginal cells of the wide pith rays are of different length. Initially they are one, then two, later on four cells long and become high tracheids. The inner cells are, however, rather isodiametric and simply pitted. On the other hand, however, rather isodiametric and simply pitted. On the other hand, the cells of pith rays of Magnoliae (Fig. 13) and generally of the higher woody.

The geographical distribution of Drimyses

The vesselless Drimyses belong to the similarly vesselless Winteraceae family. Concerning this, we can read in METCALF's book the following: "A family of trees and shrubs, occurring chiefly in certain parts of South-East Asia and South America. *Drimys* is the only genus which occurs in both the Old and New Worlds. Smith, who has recently revised the taxonomy of the family, points out that the american species are all hermaphrodites, whereas those from the Old World are dioecious (Fig. 19).

On the other hand, in ENGLER–PRANKL's paper we can already read some data in detail: "*Drimys*, Bl., zwitterig, polygam oder diklin. Ungefähr 40 Arten, davon *D. Winteri* Forst in verschiedenen Varieteten von Mexico bis zur Magellan-Strasse in den Gebirgen und höher gelegenen Gegenden, 4 Arten in Australien, 2 in Neuseeland, je 1 in Neukaledonien, Neuguinea und Borneo (Fig. 19).

But what is the connection between the geographical distribution of Drimyses, xylotomy, and the distribution of their genera? Seemingly nothing. But essentially, we can draw far-reaching conclusions from the connection of these. In order to



19. The geographical distribution of the homoxylic Drimyses. They are hermaphroditic in the whole South America (ca. 35 species), while in South-East Asia, in one or two islands, only four-five dioecious species live.

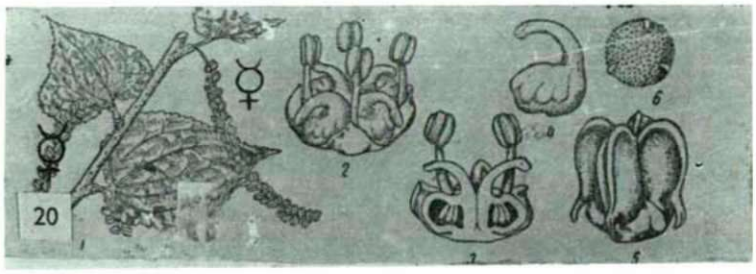


Fig. 20. The hermaphroditic amentiflorae, flower, fruit, seed, and pollen grains of *Tetracentron sinense*.

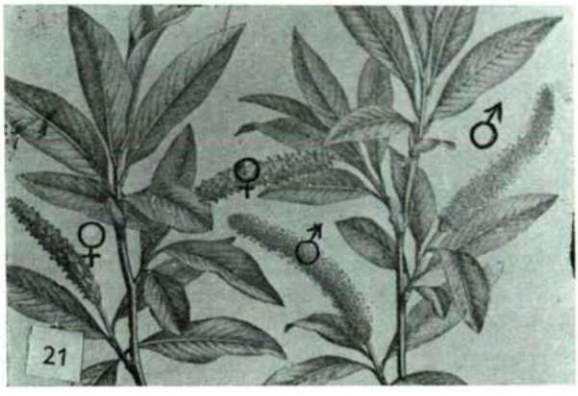


Fig. 21. The staminate and pistillate unisexual amentiflorae of the dioecious *Salix*.

understand the following, we have to mention certain basic laws of genetics. Which are these? Every living being, be it animal or plant, monocellular or gigantic, has some sex. It is, therefore, either male or female. Or, it may be hermaphroditic. To this rule, there is no exception. Thus, it concerns trees, i.e. homoxylic Drimyses, as well. Drimyses are landplants. Each individual develops from a seed. The seed, however, already contains the sexual character, as well. From one seed, only a male, i.e. a polliniferous individual develops. The plant itself is dioecious, i.e. amphibious, because the progeny, i.e. the seed needs two kinds of parents to its formation. These are the so-called homospermatic plants (Fig. 21). From the other kind of seeds, independently of its size, an individual develops, in which the two kinds of multiplying organs get nearer to each other. Thus, the creation of progeny becomes surer. In these, in certain shoots, polliniferous flowers (catkins) develop, while in other shoots female flowers. Pollination is uncertain enough in both cases because it takes place by the mediation of the wind. At any rate, this seems to be more advantageous from the point of view of the progeny than the dioecious state is.

The third type of seeds is, when from the same kind of seeds the same kinds of individuals develop. These are the most highly developed seedy land plants, angiosperms. Pollination takes here already place generally by the mediation of insects. The hermaphroditic state is therefore, more developed, though younger, than monoecism or dioecism. In the course of phylogeny, as well, the dioecious

plants with changing sex and the monoecious ones, thus Coniferae, Cycadaceae, frc., etc. appeared as first from among the high-growing plants. It is interesting that teom among the Pine families (Taxodiaceae) are rather monoecious north of the Equator, while the dioecious ones, thus Araucariaceae, Podocarpaceae, Taxaceae, Cycadaceae are mainly distributed in the southern globe.

Applying this law of nature to Drimyses, as well, among which there are both liroecious and monoecious ones and hermaphrodites, we see that among these, the dehmaphrodites are the youngest, and the dioecious ones the most ancient, i.e. the odest ones. TAKHTADJAN regards the youngest type, i.e. the Magnoliales with hermaphroditic flowers as the first in the formation of angiosperms. In our opinion, this is erroneous. For proving this, we only mention the following. Those with hermaphroditic flowers the ancient ones, then how can they arrive at the state of separated sex, dioecism? In this case, those with hermaphroditic flowers should develop instead of one seed pollen resp. individual. (Two seeds, with the organs of propagation in them, in order to develop of them healthy progeny. But this would be connected with an enormous waste of material. They would have to renounce the surer pollination by insects and choose the uncertain pollination by wind, etc., etc. Such a possibility has so far not occurred, as yet. 2) And if we consider dioecism as more ancient — as we do — we could renounce the creation of the other animal. This would, at any rate, mean economy of materials, too, but these would get, instead of the uncertain pollulation by wind, into the state of the much more certain pollulation by insects. This has followed in South America and South-East Asia, when the hermaphroditic *Drimys* species (the South-Americans in 85 p.c.) overran the latter continent, while the four to five dioecious species, having survivd ine South-East-Asia are dying out, showing that dioecism is the more ancient, older, and the hermaphroditic state the younger, more succesful and advantageous, as well.

The hermaphroditic state rather promotes the greater and faster distribution. At propagation, the hermaphroditic state and monoecism need only one, dioecism always two kinds of seeds, that is to say, two times so many. The double amount of seeds needs a double area. Therefore, distribution also needs a larger territory. Thus, it is handicapped to the monoecious and hermaphroditic individuals. And in the struggle for life, this is a very important circumstance.

The dioecious plants are, therefore, fully and necessarily pushed into the background by the hermaphroditic state, as a consequence of which the dioecious plants become more and more exhausted and perish. In the course of phylogeny, too, in the various geological ages, the species prevailed, the reproductive organs, resp. the gametes of which have got as close to one another, in space and time, as it was possible.

In the opinion of the author, it is not only improbable but even impossible, to originate the dioecism from the hermaphroditic flowers of angiosperms, because it would be opposite to evolution. The inversed process is natural. The unisexual flowers are more ancient and the bisexual ones are younger, this is verified by the palaeontological and pollenanalytical data, as well as by the enclosed Plates, too.

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FLORAL NECTARIES OF SPECIES OF PAPILIONACEAE

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Abstract

The floral nectaries of 3 varieties and 45 species of 26 genera of the family Papilionaceae were investigated. On the basis of results the following statements were made: 1. The species investigated can be ranged into 8 groups on the basis of the external morphology and anatomy of their nectaries. 2. It is possible to arrange the nectaries in a morphogenetical order according to their external appearances, whereby conclusions can be drawn concerning the phylogeny of the species of Papilionaceae. 3. The most ancient species (10 species) do not have floral nectaries, whereas those at higher stages of phylogenetic development (17 species) do. The species with automorphic floral nectary represent the highest stage of evolution (21 species). Even of them, those with zygomorphic and differentiated nectaria are regarded as the most developed ones, i.e. the youngest species. It was also possible to differentiate 6 subgroups within this latter type. It is likely that several forms of transition exist among the types listed. 4. The secretion of nectaries with greater gland volume have a higher sugar value. There is a linear correlation between the two.

Introduction

Several reports appeared in the last decades in sonnection with the structure and production of floral nectaries. Of them, however, only a few (VELENOVSKY, 1910; BROWN, 1938; GULYÁS, 1968; FAHN, 1951; GOVIL, 1975; TACINA, 1978) can be said to contain valuable information on the apicultural value, stage of development of the species of a plant family as established on the basis of the measurements and structure of sugary fluid-secreting glands. In the case of some families (Ranunculaceae, Rosaceae, Malvaceae) the occurrence and diversity in shape of the nectaries of their species can be of taxonomical phylogenetical importance (SCHWEIDLER, 1930; GREGORY, 1915; JANDA, 1937; NORRIS, 1941; DAUMANN, 1930, 1931; BROWN, 1938; WERT, 1941). BROWN (1938) claims that species with discus-type nectaries are phylogenetically related to one another. According to him Ericales-Palemoniales-Boraginales-Solanales-Personales-Laminales forms a line of relationship, and that a similar relationship exists between species with ring-shaped nectary at the base of their filaments (Caryophyllales-Polygonales-Chenopodiales). VELENOVSKY (1910) ranged the species of Cruciferae into subgroups on the basis of occurrence and number of floral nectaries. GULYÁS (1968) set up a morphogenetical order of succession within the family Labiatae.

The cultivation of such variaties and types of our culture plants, which produce nectary in greatest amount and of higher sugar content would favourably influence the honey production of bees and increase the crop because of infallible pollination.

Thus, in the knowledge of experimental results we are now in the position to foretel the nectar production of a flower — under optimal external conditions — if we know the size and structure of its nectary. In this sense, the knowledge of the morphology and anatomy of nectaries is of great significance from economical viewpoint. At the same time, investigations of this kind have also provided many new and useful taxonomical phylogenetical, etc. data for botany. The objective of thisl study was to demonstrate the apiculturally most valuable types of nectaries and their structure of those species of the economically also very important Papilionaceace (comprising about 10 000 species), which are most worth consideration in our country.

Material and Methods

For the solution of the above mentioned problems the following 3 varieties, 45 species and 26 genuses of Papilionaceae were investigated:

1. *Sophora* — *Sophora japonica* L.
2. *Lupinus* — *Lupinus albus* L.
3. *Genista* — *Genista tinctoria* L.
4. *Laburnum* — *Laburnum anagyroides* MEDIC.
5. *Cytisus* — *Cytisus albus* HACQ.; *Cytisus ciliatus* WAHLB.; *Cytisus hirsutus* L. ssp. *leucotrichus* (SCHUR) A. et G.
6. *Ononis* — *Ononis spinosa* L.
7. *Medicago* — *Medicago falcata* L.; *Medicago sativa* L.; *Medicago minima* L. DESR.
8. *Melilotus* — *Melilotus officinalis* L.
9. *Trifolium* — *Trifolium hybridum* L.; *Trifolium incarnatum* L.; *Trifolium pratense* L.; *Trifolium repens* L.; *Trifolium campestre* SCREB.; *Trifolium aureum* POLLICH.
10. *Anthyllis* — *Anthyllis vulneraria* L.
11. *Tetragonolobus* — *Tetragonolobus maritimus* (L.) ROTH ssp. *siliquosus* (L.) MURB.
12. *Lotus* — *Lotus corniculatus* L.
13. *Amorpha* — *Amorpha fruticosa* L.
14. *Galega* — *Galega officinalis* L.
15. *Robinia* — *Robinia hispida* L.; *Wistaria sinensis* (SIMS) DC.
16. *Colutea* — *Colutea arborescens* L.; *Lespedeza bicolor* TURCZ.; *Desmodium canadense* (L.) DC.
17. *Caragana* — *Caragana arborescens* LAM.; *Caragana frutex* C. KOCH.
18. *Astragalus* — *Astragalus glycyphyllus* L.
19. *Glycyrrhiza* — *Glycyrrhiza echinata* L.
20. *Coronilla* — *Coronilla varia* L.
21. *Onobrychis* — *Onobrychis viciaefolia* SCOP.
22. *Vicia* — *Vicia faba* L.; *Vicia cracca* L.; *Vicia sativa* L.
23. *Lens* — *Lens culinaris* MEDIC.
24. *Lathyrus* — *Lathyrus tuberosus* L.; *Lathyrus hyrsutus* L.

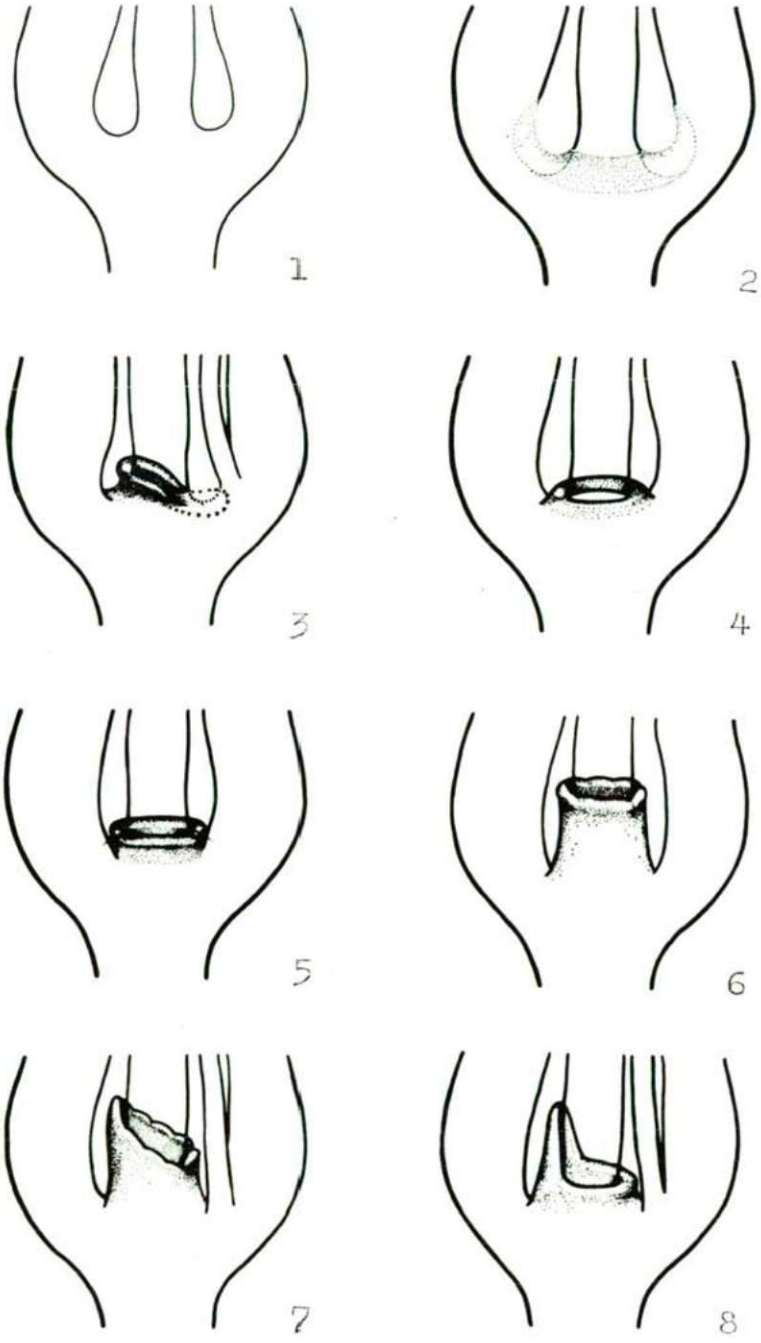


Plate I. Flower types of Papilionaceae species. Supposed morphogenetical order.

Lathyrus aphaca L.
Lathyrus odoratus L.
25. *Pisum* *Pisum sativum* L.
26. *Phaseolus* *Phaseolus coccineus* L.
Phaseolus multiflorus LAM.
Phaseolus vulgaris L. var. *communis* 'Sulphur'
Phaseolus vulgaris L. var. *nanus* 'Aranka'
Phaseolus vulgaris L. var. *communis* 'Juliska'

The floral nectaries of the above species were investigated concerning their places of occurrence, types and structure. In the course of that, correlation was sought between the measurements of the gland and nectar production in 18 cases. These studies were performed in the Botanical Garden of the Botanical Department of Attila József University, Szeged in the maximal floral season of species. Before the analysis of the sugary fluids, the flowers were covered with a 2 mm mesh-size net for 24 h, then the nectar was collected by means of thinner of thicker glass capillaries conform to the size of the flowers at 7.00 a.m. (DEMIANOWICZ and HLYM, 1960). Nectars of 200 flowers were weighed in the case of each species. The amount of nectar was determined by means of analytical resp. torsion balance, its dry matter content by using an Abbé refractometer and the sugar value of nectar on the basis of the following equation:

$$S = \frac{N}{100} \cdot \mathrm{Dm}\%$$

where S=sugar value, N=amount of nectar in mg produced in one flower during 24 hours, Dm%=dry matter.

The plant species investigated grew on ameliorated silty clay soil. Macroclimatic data during the period of investigation were recorded by the meteorological apparatuses of the Botanical Garden (relative atmospheric moisture content, temperature).

It did not belong to the scope of these studies to seek correlation between climatic changes and nectare production. The nectar yields of plants grown in similar situations and the sugar values of them were related only to their gland volumes. Values for gland volume were calculated by approximation to the most corresponding geometrical form. In the case of nectaries with a more complicate structure, the volume was approximated by division into several subforms. To make the calculation of the necessary measurements more easy, the corresponding sections of 10 glands of each species were projected on a squared plotting paper. The data were converted to mm^3. The correlation between calculated gland volume and sugar value was established by two-variable linear regression analysis (SVÁB, 1973). These examinations pertain only to the aforementioned 13 species of the following genera: *Colutea, Sophora, Caragana, Vicia, Lotus Meliotus, Onobrychis, Amorpha, Medicago* and *Trifolium*. In the case of the other species and varieties only the structure was analysed. For the investigation of structural composition, the floral nectaries were fixed in ethanol, then kept in Strassburger–Flemming preservative (SÁRKÁNY and SZALAI, 1966) until their embedding in celloidin (KISSER, 1926; ROMEIS, 1948; GULYÁS, 1968). From each nectary, 3200 longitudinal resp. transversal sections were made in the medial resp. transversal plane of section. For microscopic analysis the sections were double-stained with haematoxylin eosin and covered with Canada balsam.

Results

1. Morphology of nectaries (external morphology)

In this paper on the nectaries of some papillionacean genera FREI (1955) makes mention only of glands of epimorphic type. GULYÁS and KINCSEK (1977) ranged the flowers of the various members of Papilionaceae into 3 groups: a=species with flowers without nectary, b=those with nectary of epimorphic type, c=those having automorphic nectaries. It has been the further extending of these studies (KINCSEK, 1977), that made it possible for us to discriminate now 6 forms of automorphic nectaries (Plate I). Thus, the flowers of papilionacean species investigated by us can be ranged into 8 groups on the basis of their nectaries:

1. In the flowers of species belonging into the first group there is no nectary. These species are: *Coronilla varia* L., *Cytisus albus* HACQ., *Galega officinalis* L., *Genista tinctoria* L., *Laburnum anagyroides* MEDIC., *Ononis spinosa* L., *Lupinus albus* L., *Trifolium aureum* POLLICH., *Tetragonolobus maritimus* (L.) ROTH, ssp. *siliquosus* (L.) MURB., *Desmodium canadense* (L.) DC. These together make 20.83% of the species investigated.

2. Nectary of epimorphic type, located around the base of the gynoecium in the inner side of the receptacle: *Sophora japonica* L., *Cytisus ciliatus* WAHLB., *Medicago falcata* L., M. *sativa* L., M. *minima* L., *Trifolium hybridum* L., *Anthyllis vulneraria* L., *Lotus corniculatus* L., *Amorpha fruticosa* L., *Colutea arborescens* L., *Caragana arborescens* LAM., C. *frutex* C. KOCH., *Glycyrrhiza echinata* L., *Vicia faba* L., *Lens culinaris* MEDIC., *Lathyrus tuberosus* L., *Pisum sativum* L. These constitute 35.4% of the investigated species.

3. One part of the nectary is epimorphic, the other part localited opposite to the 10 free stamens forms a ring-like protrusion: *Trifolium campestre* SCHREB., *Astragalus glycyphyllus* L., *Lathyrus odoratus* L., *L. hirsutus* L. These make 8.3% of the investigated species.

4. The nectary is flattened ring-shaped, i.e. automorphic. *Cytisus hirsutus* L. ssp. *leucotrichus* (SCHUR) A. et G., *Melilotus officinalis* L., *Lathyrus aphaca* L. These constitute 6.2% of the investigated species.

5. Nectary automorphic, surrounding ring-like the base of the ovary. *Trifolium pratense* L., *T. repens* L., *T. incarnatum* L., *Onobrychis viciaefolia* SCOP., *Vicia cracca* L. These constitute 10.4% of the investigated species.

6. The nectary surrounds pipe-like the base of the gynoecium. It is automorphic. Robinia hispida L., *Wistaria sinensis* (SIMS) DC., *Lespedeza bicolor* TURCZ. These represent 6.2% of the investigated species.

Plate II. *Phaseolus multiflorus* LAM. (x17)
Phaseolus vulgaris L. var. *com.* "Juliska" (x15)
N = Nectary

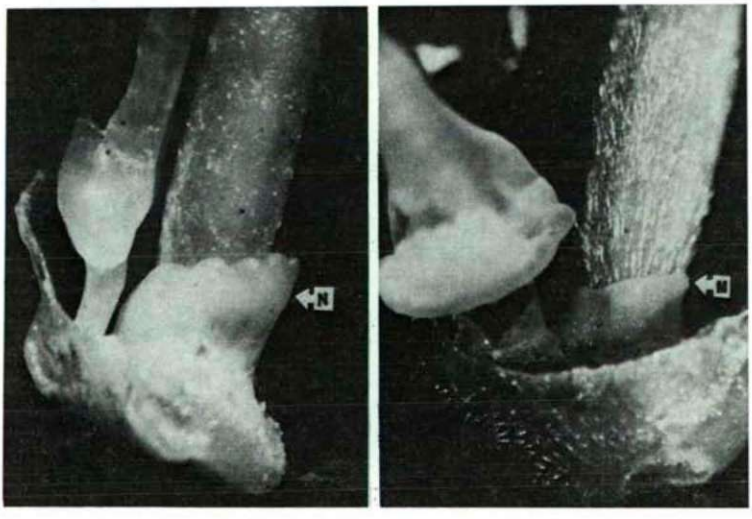
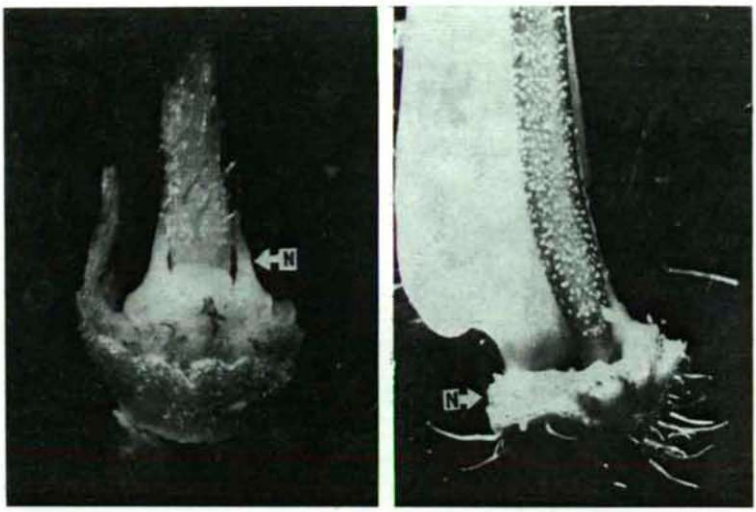


Plate III. *Phaseolus coccineus* L. (x25) *Robinia hispida* L. (x8) N = Nectary



7. The nectary is pipe-like, but on the side of the free stamens it is lower, automorphic. *Phaseolus coccineus* L., *P. multiflorus* LAM., *P. vulgaris* L. var. *communis* "Sulphur", *P. vulgaris* L. var. *nanus*, "Aranka", *P. vulgaris* L. var. *communis* "Juliska". 10.4% of the investigated species belong there (Plate II., III).

8. The part of the ring-shaped automorphic nectaries opposite to the tenth free stamen is tongue-like elongated. *Vicia sativa* L. This constitutes 2.0% of the investigate species.

The results reported here show that the nectaries of the species of Papilionaceae are most varied in shape. It is also possible to arrange the various nectaries in a morphogenetical order of sequence. Within the family, those species can be regarded as the most ancient ones which have no nectaries. In the more developed forms epimorphic nectaries appear in the flowers. The next stage of development is represented by species with automorphic nectaries, though here the nectaries still exhibit radial symmetry.

Those species of the Papilionaceae are the most developed ones which have zygomorphic automorphic nectaries. The differentiation of developmental stages on the basis of nectaries is not only possible within the families, but within genera of great species number, too. E.g., the three *Cytisus* species represent 3 stages of development. Similarly, 3 developmental levels can be differentiated in the genera *Colutea, Vicia* and *Lathyrus*. On the other hand, in the case of *Trifolium*, besides

Plate IV. Longitudinal sections of nectaries
1. *Lathyrus aphaca* L. (x70)
2. *Glycyrrhiza echinata* L. (x200)
3. *Colutea arborescens* L. (x110)
4. *Vicia cracca* L. (x190)
5. *Lathyrus odoratus* L. (x85)

Plate IV

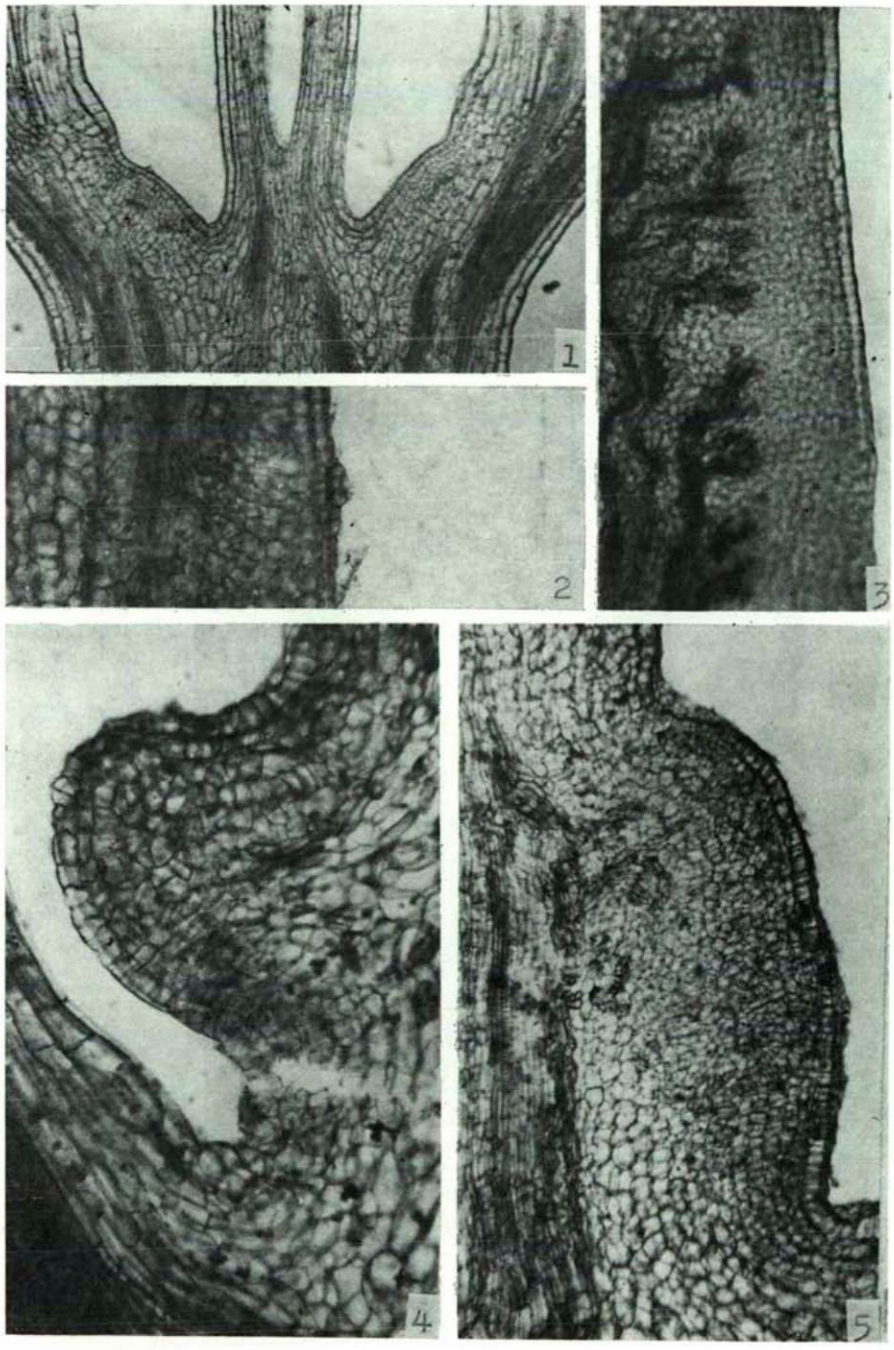


Table 1. Vascular bundles of nectaries

Name	Vascular bundles		
	no bundles	phloem	phloem + xylem
1. *Sophora japonica* L.	–		
2. *Cytisus ciliatus* WAHLB.	–		
3. *Medicago falcata* L.			+
4. *Medicago sativa* L.	–		
5. *Medicago minima* L. DESR.	–		
6. *Trifolium hybridum* L.	–		
7. *Anthyllis vulneraria* L.	–		
8. *Lotus corniculatus* L.	–		
9. *Amorpha fruticosa* L.	–		
10. *Colutea arborescens* LAM.		+	
11. *Caragana arborescens* LAM.		+	
12. *Caragana frutex* C. KOCH.	–		
13. *Glycyrrhiza echinata* L.	–		
14. *Vicia faba* L.	–		
15. *Lens culinaris* MEDIC.	–		
16. *Lathyrus tuberosus* L.		+	
17. *Pisum sativum* L.	–		
18. *Trifolium campestre* SCREB.	–		
19. *Astragalus glycyphyllus* L.	–		
20. *Lathyrus odoratus* L.		+	
21. *Lathyrus hyrsutus* L.		+	
22. *Cytisus hyrsutus* L. ssp. *leucotrichus* (SCHUR) A. et G.	–		
23. *Melilotus officinalis* L.	–		
24. *Lathyrus aphaca* L.		+	
25. *Trifolium pratense* L.	–		
26. *Trifolium repens* L.	–		
27. *Trifolium incarnatum* L.	–		
28. *Onobrychis viciaefolia* SCOP.		+	
29. *Vicia cracca* L.		+	
30. *Robinia hispida* L.		+	
31. *Wistaria sinensis* (SIMS) DC.		+	
32. *Lespedeza bicolor* TURCZ.		+	
33. *Phaseolus coccineus* L.		+	
34. *Phaseolus multiflorus* LAM.		+	
35. *Phaseolus vulgaris* L. var. *communis*, Juliska,			+
36. *Phaseolus vulgaris* L. var. *communis*, Sulphur,			+
37. *Phaseolus vulgaris* L. var. *nanus*, Aranka			+
38. *Vicia sativa* L.			+

species with 3 different types of nectaries, those without nectaries are also found. Thus this genus exhibiting 4 different developmental stages can be regarded as the most diversified one (Plate IV).

Structure of the nectary

Concerning the structure of nectaries it is known that their glandular tissue is of epidermal origin and is made up of parenchyma and vascular bundles. Quality and quantity of the produced sugary fluid are determined by the number and composition of the vascular bundles and the size of the gland. According to FREI (1955)

the vascular bundles, in case they occur in the nectaries of Papilionaceae, are made up solely of phloem elements. Own investigations have shown that 44% of the nectaries of papilionacean species contain vascular bundles. Of them 34% contain only phloem elements, 10% xylem and phloem elements together. It is known that glands supplied with vascular bundles always produce sugary fluid in abundance.

Table 2. Volumes and sugar values of nectaries

Name of species	Volume of nectary X(mm³)	Sugar value Y(mg)
1. *Colutea arborescens*	0.653	0.90
2. *Sophora japonica*	0.525	0.98
3. *Caragana arborescens*	0.322	0.40
4. *Caragana frutex*	0.213	0.50
5. *Vicia cracca*	0.189	0.10 (KARTASOVA, 1957)
6. *Lotus corniculatus*	0.151	0.30 (BEUTI ER, 1941; KUI IEV, 1952; PÉTER, 1971; KUBISOVA-KROPACOVA and NEDBALOVA, 1975)
7. *Melilotus officinalis*	0.099	0.12 (KULIEV, 1952)
8. *Onobrychis viciaefolia*	0.067	0.21 (PÉTER, 1971)
9. *Amorpha fruticosa*	0.038	0.11 (PÉTER, 1971)
10. *Trifolium repens*	0.008	0.15 (MAURIZIO, 1958; PÉTER, 1972)
11. *Medicago sativa*	0.006	0.09 (GLUKOV, 1950; KROPACOVA, 1960; PÉTER, 1972)
12. *Trifolium pratense*	0.005	0.13 (OSZTASCSENKO and KUDRJACEVA, 1956; PÉTER, 1971)
13. *Trifolium campestre*	0.002	0.07 (KULIEV, 1952)

After the sugar values not determined by ourselves the literary source is indicated. If in the case of the same species more than one literary data were available, the averages of data were taken into consideration. The following correlation was

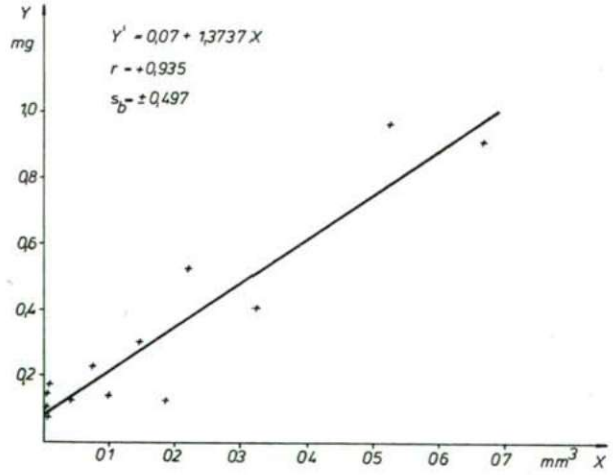


Fig. 1. Correlation between gland volume and the sugar value of nectar produced.

found between calculated gland volumes (mm^3) and weighed sugar values (mg) resp. sugar values taken over from literature. Nectaries of greater capacity have greater sugar values. There is a linear correlation between the two (Fig. 1). The regression coefficient of the linear regression equation shows that e.g. 1 mm^3 gland produces 1.3737 mg sugar during 24 hours. This value is true in the interval $x=0.002–0.7$.

On principle, a saturation graph assures a much better approximation of the relationship between gland volume and sugar value. However, to do this the number of $x-y$ should be increased, resp. extended to the interval beyond the value $x=0.7$. The saturational relationship can be explained by the fact that with the increasing of glandular volume the ratio between glandular tissue and glandular parenchyma gradually decreases. It is the task of further investigations to verify this.

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STUDIES ON THE POLLEN GRAINS OF RECENT CASTANEOIDEAE. I

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Abstract

Pollen grains of the genera *Castanea, Castanopsis, Chrysophylla* and *Pasania* were investigated. Light microscopically almost all pollen grains were psilate, tricolporate. On the basis of light microscopic features, it was possible to differentiate various types which can be also used to establish botanical relationships of fossil forms. The Castaneoideae pollen grains were in many cases similar to the pollen of the genus *Elaeocarpus*. Lesser or greater similarities can occur also with other taxa, which should be considered in the judgement of the botanical relationships of the fossil pollen grains of Castaneoideae. Several problems can be only solved by electron microscopic methods.

Introduction

According to POTONIÉ's (1931, 1934) basic works and the monograph by THOMSON and PFLUG (1953) our knowledge of fossil pollen grains of Castaneoideae ranges from the Cretaceous period to the Upper Tertiary. Its modern nomenclature has been termed by POTONIÉ (1960). Many other works contributed to the knowledge of these pollen grains, e.g. TRAVERSE (1955), AGRANOVSKAIA et al. (1960) and KEDVES (1963). Transmission electron microscopic (TEM) data originate from KEDVES and PÁRDUTZ (1973) as well as CREPET and DAGHLIAN (1980).

It is indispensable to consider also data on pertaining recent forms to make our knowledge of fossil forms accurate. A number of literary data are available on the recent pollen grains of Castaneoideae. THANIKAIMONI (1972) mentions 13 literary data on *Castanea*, 10 on *Castanopsis* and 2 on *Chrysolepis*. WANG et al. (1960) reported data on the light microscopic morphology of the pollen grains of the genera *Castanea, Castanopsis, Lithocarpus* and *Pasania*, while KUPRIANOVA (1965) on those of *Cyclobalanopsis, Trigonobalanus, Castanea, Lithocarpus, Castanopsis* and *Chrysolepis*. The first data on the scanning electron microscopic (SEM) morphology of *Lithocarpus densifolia* are known from MARTIN and DREW (1969). HUANG (1972) published data on *Castanopsis, Cyclobalanopsis, Lithocarpus* and *Pasania*. In the course of his studies on pollenkitt, HESSE (1978) also investigated the TEM ultrastructure of *Castanea sativa*. Worthy of mention are LIEUX's (1980) recent SEM results and PRAGLOWSKI's (1980) following statement: "Castaneoideae are rather stenopalynous, while two entirely different pollen types are obvious in the Fagoideae and Quercoideae".

The following problems should be stressed here on the basis of available literature:

1. Intergeneral similitudes can occur also within Castaneoideae, moreover,

some of the pollen grains cannot be easily distinguished from those of Elaeocarpaceae *(Elaeocarpus)* by light microscopy. Palynological similitude was observed among other families to Araliaceae *(Anomopanax schlechteri)*, Crassulaceae *(Sedum tatarinowii)*, Elatinaceae *(Elatine alsinastrum)*, Grubbiaceae *(Grubbia rosmarinifolia)*, Leguminosae *(Sophora japonica)*, Loganiaceae, Buddlejovideae.

2. In the light of recent data, the fossil taxons — form-species — are heterogeneous.

It is these problems, that called for complex investigations. These are in process now. This paper, which forms the first chapter of these investigations approaches the question by the limited possibilities of the light microscopic method. Despite that, however, these studies are also necessary, because this method is currently used for the routine investigation of fossil materials. Our knowledge in this field will be complemented later with TEM and SEM data.

Materials and Methods

The material of investigation was made available from the Botanical Collection of the Hungarian Natural History Museum by the courtesy of Director JULIA SZUJKÓ, for which I express my grateful thanks also here. After the name of the species investigated the number of that herbarium sheet is given from which the sample was taken.

Castanea americana RAF. = *C. dentata* BORCKH. 77101, *C. evansii* ELM., 77105, *C. pumila* MILL. var. *angustifolia* 77132, *C. sativa* MILL. 77047, *Pasania calathiformis* (SKAN.) H. et C. 286417, *P. hypoglauca* HU 286499, *Castanopsis argyrophylla* KING, 77112, *C. indica* DC. 7721, *C. longispicata* HU 77121, *Chrysolepis chrysophylla* (A. DC.) HJELMQVIST 77093.

For variation analysis at least 200 grains were measured. The longitudinal axis resp. the ratio of the longitudinal axis to the meridional one were taken into consideration.

Results

Genus: *Castanea* MILL.

1. *Castanea americana* RAF. (Plate I, 1–6).

Psilate, tricolporate pollen grains, amb elliptical. Colpi bending towards one another at the poles. Colpi surrounded by narrow caverns. Endopori ellipsoid, measuring 2 μm on the average. Longitudinal axis measures 11.25 μm to 19.55 μm. Its maximum is protracted, between 14.7 μm and 16.65 μm. The meridional axis varies from 8.85μ m, to 16.15 μm, with a maximum at 9.8 μm. The ratio of the longitudinal axis to the meridional one is between 1 and 1.9, maximum is 1.5–1.6. Exine 1 μm thick, structure difficult to recognize, perhaps intrabaculate.

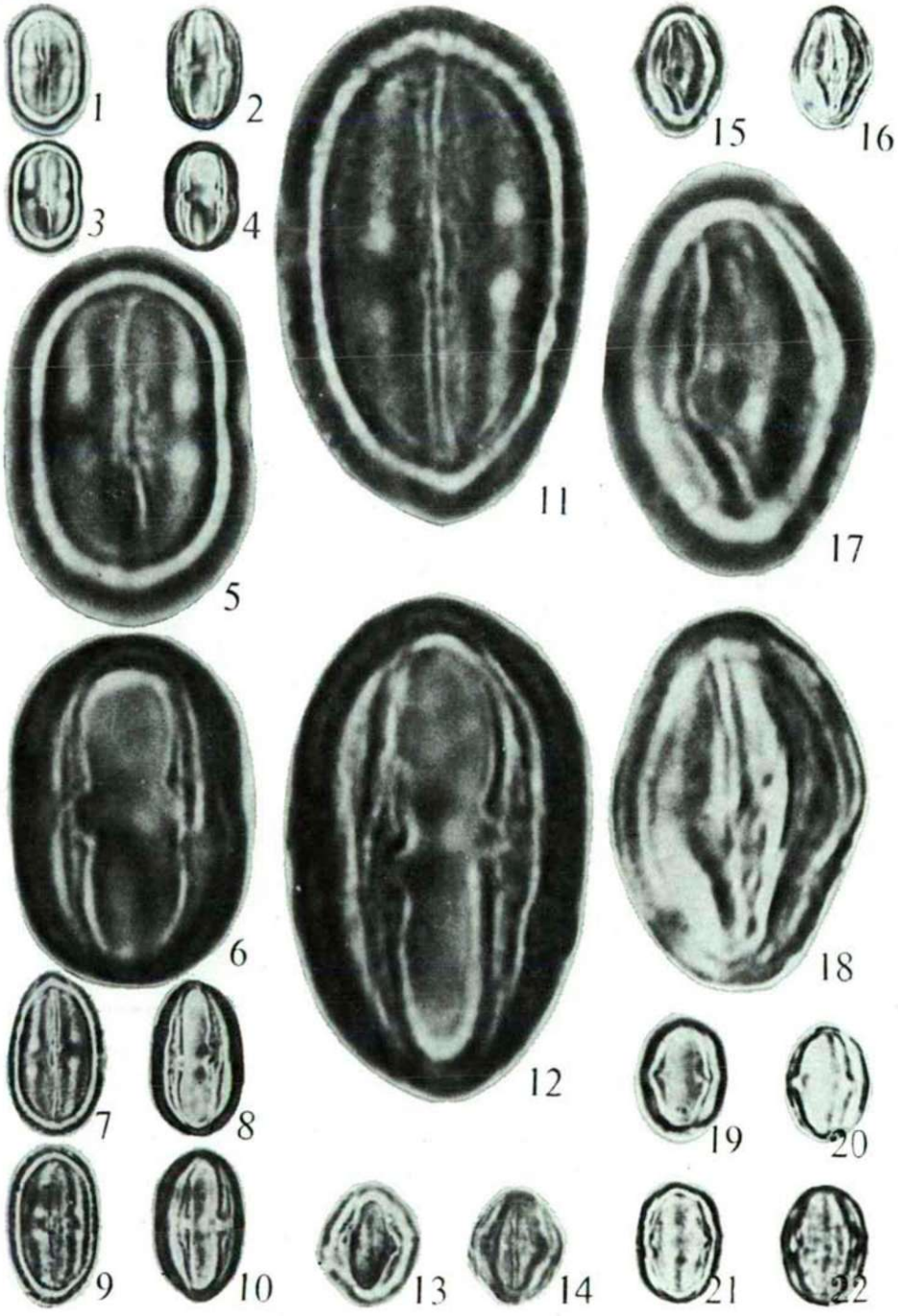
2. *Castanea evansii* ELM. (Plate I. 7–12).

Tricolporate pollen grains, amb elliptical, surface psilate. Colpi bent towards one another at the poles and surrounded by narrow (0.3–0.4 μm) caverns. Endopori

Plate I

1—4. *Castanea americana* **Raf.** x1000.
5, 6. *Castanea americana* **Raf.** x3000.
7—10. *Castanea evansii* **Elm.** x1000.
11, 12. *Castanea evansii* **Elm.** x3000.
13—16. *Castanea pumila* **Mill.** var. *angustifolia* x1000.
17, 18. *Castanea pumila* **Mill.** var. *angustifolia* x3000.
19—22. *Castanea sativa* **Mill.** x1000.

Plate I



circular or meridional ellipsoid, 1.5 μm in diameter on the average. Longitudinal axis varies from 18.7 to 23.4 μm, maximal length is 20.66 μm. Meridional axis measures 9.8 μm to 13.7 μm, with a maximum of 11.2 μm–13.2 μm. The ratio of the longitudinal axis to the meridional one is 1.1–2.2, with a maximum between 1.5 and 2.0. Exine 1 μm in diameter, infratectum more distinct than in the case of the former species, finely intrabaculate.

3. *Castanea pumila* MILL. var. *angustifolia* (Plate I, 13–18).

Pollen grains ellipsoidal, or approx isodiametric psilate, tricolporate. The caverns surrounding the colpi are very narrow, often missing. Colpi not always bent towards one another at the poles. Endopores very small, pollen grains often poroidate and not tricolporate. Endopores measure 0.5 μm on the average. Longitudinal axis varies from 14.2 μm to 20.05 μm, with a maximum at 14.7 μm. Meridional axis varies from 7.15 μm to 16.65 μm, its maximum is at 14.7 μm. The ratio of longitudinal axis to the meridional one is between 1.0 and 1.9, the maximum between 1.2 and 1.4. Exine always below 1 μm in thickness, the structure is difficult to observe under the light microscope.

4. *Castanea sativa* MILL. (Plate I, 19–22, Plate II, 1, 2).

Psilate, tricolporate pollen grains, amb elliptical. Colpi bending towards one another at the poles. Caverns surrounding the colpi very narrow, but distinct, generally 0.5 μm wide. Endopores 1–1.5 μm in diameter, mostly circular. Longitudinal axis varies from 11.25 μm to 17.15 μm, the meridional one from 7.0 μm to 14.7 μm, with maxima of 17.7 μm resp. 10.75 μm. Ratio of longitudinal axis to the meridional one ranges from 1 to 2.1, maximum at 1.3. Thickness of exine always below 1 μm, exine stratification is not distinctly observable, structure uncertain on the basis of light microscopic data.

Genus: *Pasania* (MIG.) OERSTED = *Lithocarpus* BLUME.

1. *Pasania calathiformis* (SKAN.) H. et C. (Plate II, 3–10).

Psilate, tricolporate pollen grains, amb elliptical. Colpi usually surrounded by 0.3 μm wide caverns. Colpi bent towards one another at the poles. Endopores of variable size and shape, circular or elliptical, 1–2.5 μm. Longitudinal axis varies from 11.25 to 20 μm, protracted maximum from 15.15 μm to 19.5 μm. Meridional axis ranges from 8.35 μm to 13.2 μm, maximum is 9.8 μm. Ratio of longitudinal axis to meridional one from 1.2 to 2.2, maximum 1.8. Exine 1–1.2 μm thick, finer structure is not visible under the light microscope.

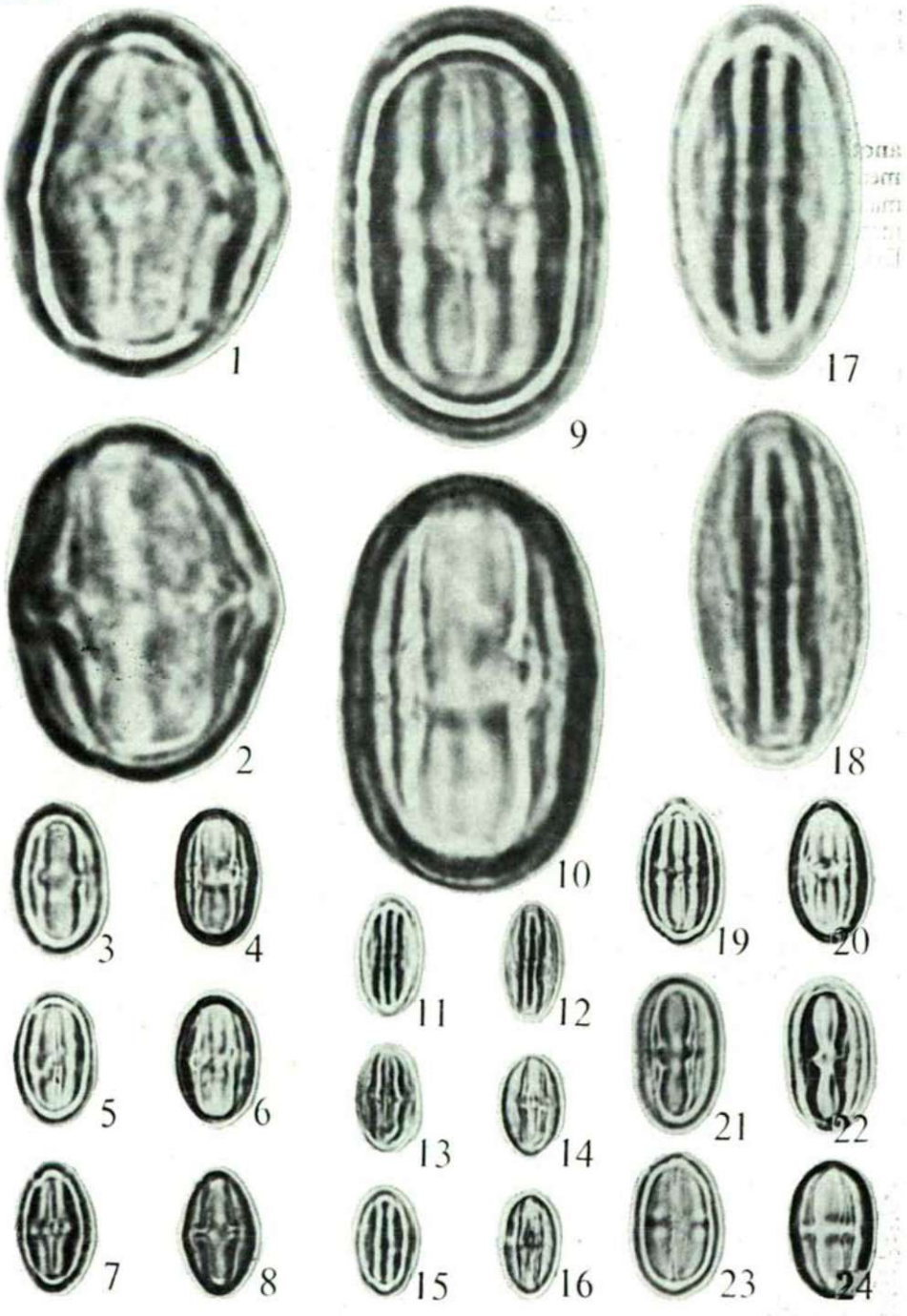
3. *Pasania hypoglauca* HU (Plate II, 11–18).

Tricolporate pollen grains, amb elliptical, surface psilate. Colpi often parallel, but bending towards one another at the poles. Caverns around colpi 0.3 μm wide. Endopores narrow elliptical, measuring 0.5×1.5 μm. Longitudinal axis varies from 11.7 μm to 15.2 μm, maximum at 14.55 μm. Meridional axis 6.6–10.3 μm, maximum at 8.35 μm. Ratio of longitudinal axis to the meridional one varies from 1.3 to 2.3,

Plate II

1, 2. *Castanea sativa* **Mill.** x3000.
3—8. *Pasania calathiformis* **(Skan). H.** et **C.** x1000.
9, 10. *Pasania calathiformis* **(Skan.) H.** et **C.** x3000.
11—16. *Pasania hypoglauca* **Hu** x1000.
17, 18. *Pasania hypoglauca* **Hu** x3000.
19—24. *Castanopsis argyrophylla* **King** x1000.

Plate II



maximum is protracted, from 1.5 to 1.8. Exine about 0.8 μm thick, stratification is not observable under the light microscope.

Genus: *Castanopsis* (D. DON) SPACH.

1. *Castanopsis argyrophylla* KING (Plate II, 19–24, Plate III, 1, 2).

Psilate, tricolporate pollen grains, amb elliptical. Colpi bent towards one another at the poles. Caverns around colpi 0.3–0.4 μm wide. Endopores elliptical, measuring 1 to 1.5×2 to 2.5 μm. Longitudinal axis varies from 14.5 μm to 22.9 μm, maximum at 19.5 μm. Meridional axis ranges from 8.85 μm to 13.3 μm, with a maximum at 10.75 μm. Ratio of longitudinal axis to the meridional one is 1.4–1.8–2.1. Exine approx 1 μm thick, finer stratification is not visible under the light microscope.

2. *Castanopsis indica* DC. (Plate III, 3–8).

Psilate, tricolporate pollen grains, amb elliptical. Colpi bent towards one another. Caverns surrounding colpi vary from 0.2 μm to 0.3 μm in width. Endopori circular or elliptical, measuring maximally 2–3 μm. Longitudinal axis varies from 17.1 μm to 23.4 μm, with two maxima, one at 19.5 μm and another at 21.05 μm Meridional axis is 8.85 μm to 13.7 μm, maximal length 11.25 μm. The ratio of the longitudinal axis to the meridional one ranges from 1.4 to 2.3, with a maximum at 1.8. Exine about 1 μm thick, its finer stratification is not visible under the light microscope.

3. *Castanopsis longispicata* HU (Plate III, 9–14).

Psilate, tricolporate pollen grains, amb elliptical. Colpi often parallel, not always bent towards one another at the poles. Caverns surrounding colpi 0.4 μm wide. Endopori approx circular, 2–3 μm in diameter. Longitudinal axis measures 17.0 μm to 23.85 μm, maximum at 19.5 μm resp. 20.95 μm. Meridional axis varies from 9.8 μm to 17.05 μm, maximum between 10.75 and 12.7 μm. The ratio of the longitudinal axis to the meridional one is 1.1–1.7–2.0. Exine approx 1 μm thick, its fine stratification is not visible under the light microscope.

Genus: *Chrysolepis* HJELMQVIST.

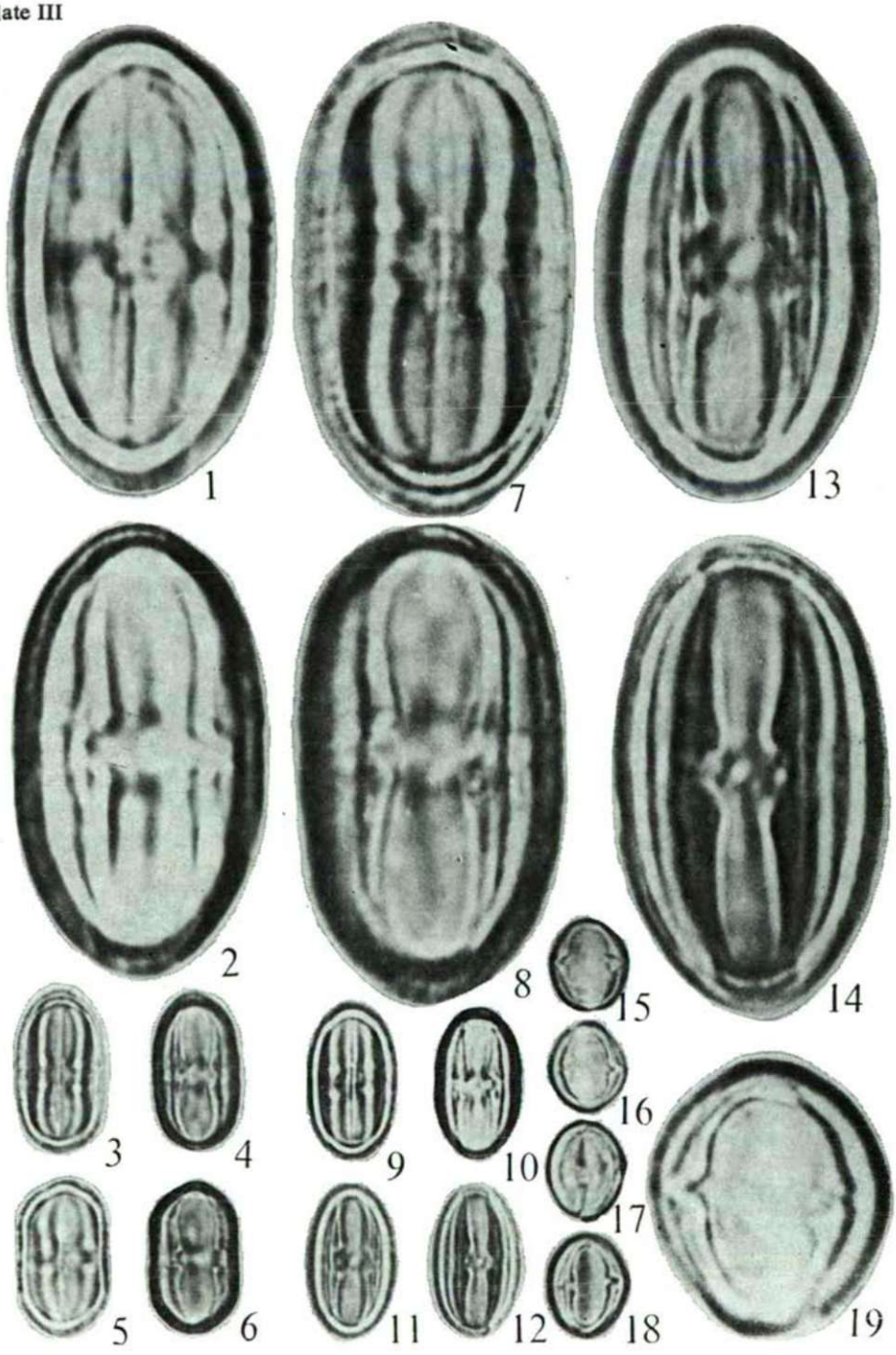
1. *Chrysolepis chrysophylla* (A.DC.) HJELMQVIST (Plate III, 15–19).

Psilate, tricolporate pollen grains, amb circular or elliptical. Colpi often parallel, convergent at the poles. Caverns surrounding colpi very narrow, often missing. Longitudinal axis measures 8.8 μm to 15.6 μm, with a protracted maximum from 13.2 μm to 14.7 μm. Meridional axis measures 7.9 μm to 12.2 μm, with a projecting maximum at 9.8 μm. Ratio of the longitudinal axis to the meridional one is 1–1.3–1.8. Exine thin, approx 0.6 μm thick, its finer structure cannot be observed under the light microscope.

Plate III

1, 2. *Castanophis argyrophylla* **King** x3000.
3—6. *Castanopsis indica* **DC.** x1000.
7, 8. *Castanopsis indica* **DC.** x3000.
9—12 *Castonopsis longispicata* **Hu** x1000.
13, 14. *Castanopsis longispicata* **Hu** x3000.
15—18. *Chrysolepis chrysophylla* **(A. DC.) Hjelmqvist** x1000.
19. *Chrysolepis chrysophylla* **(A. DC.) Hjelmqvist** x3000.

Plate III



Discussion

It should be emphasized also here that the light microscopic investigations will be complemented with TEM and SEM data in the next future. The possibilities and limitations of the single methods emerged also in connection with the present investigations. The light microscopic method has the indisputable advantage of allowing mass analyses which are indispensable with fossil forms in many respects. On the other hand, light microscopic studies leave several questions open. E.g.

1. The fine ornamentation of tectum, which may perhaps be of differential value in respect of the pollen grains of similar morphology of Elaeocarpaceae and those of further taxons, cannot be resolved. Thus, practically, all pollen grains examined qualify as psilate.

2. The light microscopic method cannot be used to investigate the stratification of exine, the morphology of the channels of tectum, infratectum, the presence and submicroscopic morphology of endexine. In the case of fossil pollen grains of Castaneoideae KEDVES and PÁRDUTZ (1973) revealed the granular ultrastructure of endexine.

The results reported here can be useful in differentiating some types on the basis of measurements, form and the structure of endopori as well as the evaluation of fossil data. Taking into consideration HUANG's data (1972) two types were differentiated within Elaeocarpaceae: 1. *Elaeocarpus arthropus (E. caroliensis, E. decipiens)*, 2. *E. japonicus* and *Elaeocarpus joga* Merril (LEOPOLD, 1969), which is reminiscent of the pollen grains of Castaneoideae, and which should be considered in the judgement of fossil forms. In possession of our data the following types were established:

1. *Castanea americana* type *(C. evansii, Pasania calathiformis, Castanopsis argyrophylla, C. indica, C. longispicata)*. It resembles *Elaeocarpus japonicus*. Its fossil equivalent is Cupuliferoipollenites pusillus (R. POT. 1934) R. POT. 1960.

2. *Castanea pumila angustifolia* type. Here the poroidate character has differentiating value.

3. *Castanea sativa* type. Its fossil equivalent is Cupuliferoipollenites oviformis (R. POT. 1931) R. POT. 1960.

4. *Chrysolepis chrysophylla* type. It differentiates from the former by its smaller measurements and nearly globular forms. Its fossil equivalent is the smaller specimen of *C. oviformis*. On the other hand, this resembles best *Elaeocarpus arthropus* type pollen grains.

Finally attention should be called here also to similarities among further taxa mentioned in the introduction. For their accurate evaluation, however, EM, principally SEM methods are necessary.

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COMPLEX STUDIES ON THE POLLEN GRAINS OF ELAEAGNUS ANGUSTIFOLIA L.

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Abstract

Pollen grains of different maturity as well as acetolyzed and non-acetolyzed ones of *Elaeagnus angustifolia* L. were investigated under light, transmission resp. scanning electron microscope. During the investigation of the diameters of pollen grains the Cushing effect was also taken into consideration. Qualitative results revealed tetraexitus and plicate forms, too. This and the brevicolporate character with vestibulum are the most reminiscent of the ancien Normapolles, the *Complexiopollis* W. KR. 1959 em. TSCHUDY 1973. Further identities are the lamellar foot layer in the germinal region and the rugulate-corrugate surface.

Introduction

There is abundant literature on the pollen grains of the genus *Elaeagnus*. These were reviewed by THANIKAIMONI (1972). Earlier reports (AMBRUSTER and JACOBS, 1934; ERDTMAN, 1954, 1966, WANG FU-HSIUNG, CHIEN NAN-FEN, YANG HUI-QU and ZHANG YU-LONG, 1960; ERDTMAN, BERGLUND and PRAGLOWSKI, 1961; GUINET, 1962; HUANG, 1972; KUPRIANOVA and ALYOSHINA, 1972; MC ANDREWS, BERTI and NORRIS, 1973) have shown that the pollen grains of the *Elaeagnus* possess such ancient properties, which surpass the primitive character of the recent pollen grains of Amentiflorae. Fossil data (e.g. KRUTZSCH, 1962; GRAY, 1964; GRUAS-CAVAGNETTO; 1978) are also supportive of the significance of the pollen grains of Elaeagnaceae.

Pollen grains of *Boehlensipollis* W. KR. 1962 and the form-genus *Slowakipollis* W. KR. were related to Elaeagnaceae by several authors. (POKROVSKAYA and STELMAK, 1960; TIMOSINA 1965; POLUMISKOVA et al. 1966; BOITSOVA and POKROVSKAYA 1966; BLYAKHOVA, 1971; HOCHULI, 1978; CHATEAUNEUF, 1980; OLLIVIERRE-PIERRE, 1980). Further fossil form-genuses are: *Elaeagnacites* KE et SHI 1978 (in SUNG TZE CHEN et TSAO LIU), *Elaeagnuspollenites* HUANG 1980. Concerning recent taxons of the family only LEINS' (1967) TEM data are known. Detailed literature, particularly on the TEM structure of the germinal aperture is not available yet. In view of the phylogenetical value of pollen grains, it was thought justified to investigate them thoroughly. The studies on the pollen grains of Elaeagnus angustifolia L. were performed to

1. investigate quantitatively and qualitatively the pollen grains at two different levels of ontogenesis,
2. investigate the Cushing effect on pollen grain preparations by various methods,

3. establish the general ultrastructural and ultrasculptural properties,

4. evaluate TEM and SEM features in the function of the maturity of pollen grains and the methods applied.

Materials and Methods

The material investigated was collected by Z. SZABÓ in the Botanical Garden of Attila József University, Szeged during spring, 1979. Pollen sacs of mature resp. closed buds were removed and used in acetolyzed resp. non-acetolyzed condition for light, transmission and scanning electron microscopic investigations. For light microscopy 39.6% hydrated glycerin-jelly was used according to LOBREAU (1966). To investigate the variation of diameter, 200–250 pollen grains at both stages of maturity and prepared by each of the above methods were measured. For the investigation of Cushing effect, the measurements performed immediately after the preparations were repeated in December, 1979. For TEM studies, the material was fixed with OsO_4 (distilled water) and embedded into araldite. Ultrathin sections were cut with a glass knife. JEOL-100B electron microscope was used for examination and for taking micrographs. Its resolving power was 2Å. For SEM studies, the pollen grains were mounted on polyvinil-chloride adhesive-coated grids and evaporated with gold-palladium alloy. The fine sculpture was studied with the JEM-ASID scanning adapter of the aforementioned apparatus.

Results

1. Light microscopic results

The surface, contour and the nature of the germinal aperture of the pollen grain was not changed by the method used for the preparation of the material. The equatorial contour was triangular, with convex sides, the germinal region prominent. Surface finely rugulate-corrugate. Colpus narrow, 18–20 µm long (Plate I, 1, 2), vestibulum marked, endoaperture pore (Plate II, 1–7). Plicae, but principally pseudoplicae, as well as tetraexitus forms occasionally occurred (Plate I, 3–8). In the case of tetraexitus forms the contour was generally convex.

On the basis of the variation of measurements the following table was composed:

	October			December		
	measurements in					
	l	u	m	l	u	m
Mature flower, non-acetolyzed	30.0–60.4		44.1	39.0–60.4		44.0
Bud, non-acetolyzed	35.2–50.0		44.0	38.5–50.0		44.0
Mature flower, acetolyzed	34.7–56.4		49.0	38.0–62.0		49.0
Bud, acetolyzed	30.2–52.4		49.0	40.1–55.4		49.0

(l = lower, u = upper, m = maximum; the greatest and the smallest pollen grain, and the measurements occurring in maximal amount, in µm).

From the data the following conclusions were drown:

1. The measurements of pollen grains prepared by the same method were approx identical in the same time point, meaning that the pollen grains of the closed bud and those of the open flower do not differ from each other in this regard.

Plate I. Light microscopic picture of the pollen grains of *Elaeagnus angustifolia* L. x1000.
1, 2 typical specimen.
3–6. plicate forms.
7, 8 tetraexitus form.

Plate I

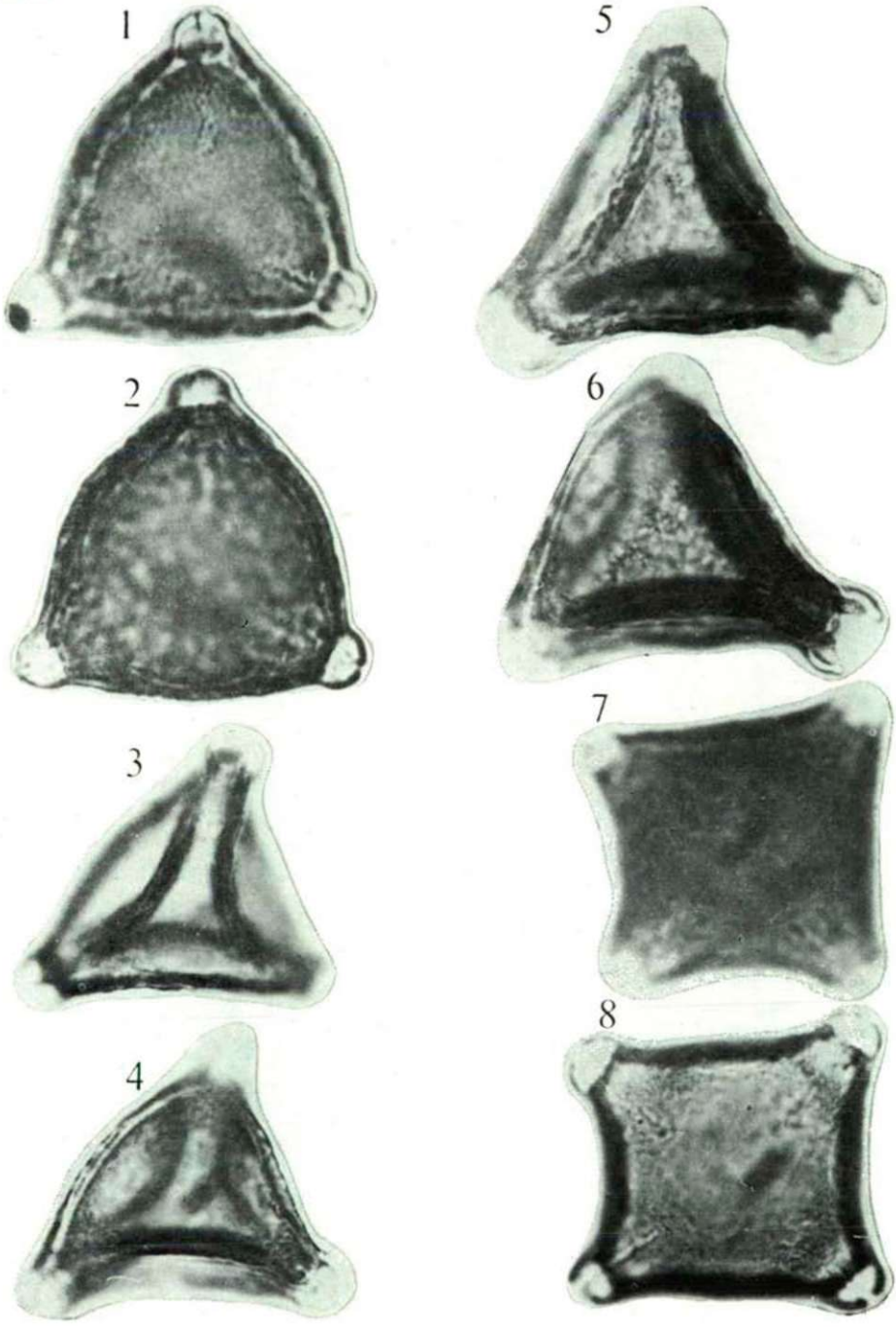


Plate II

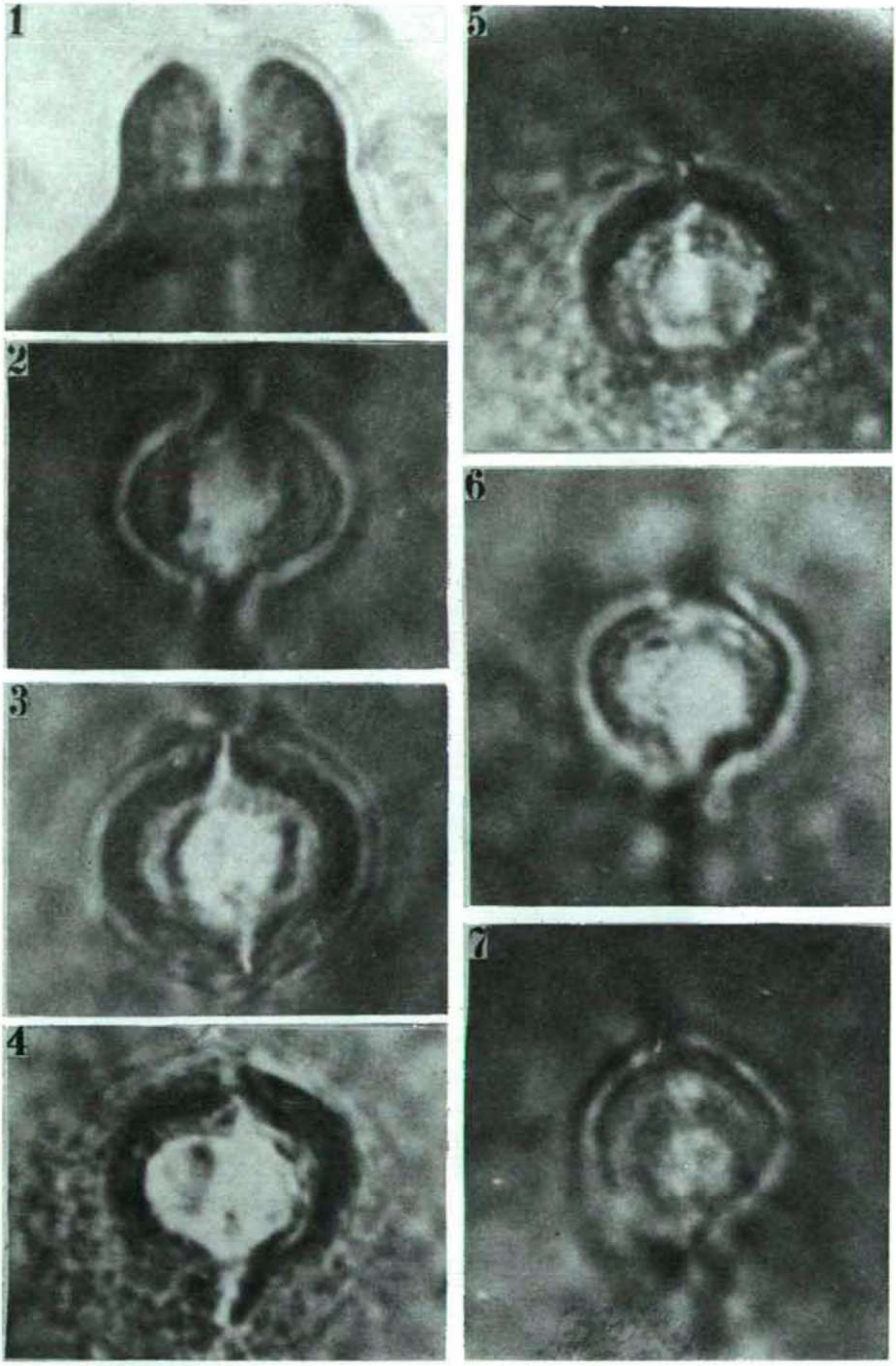
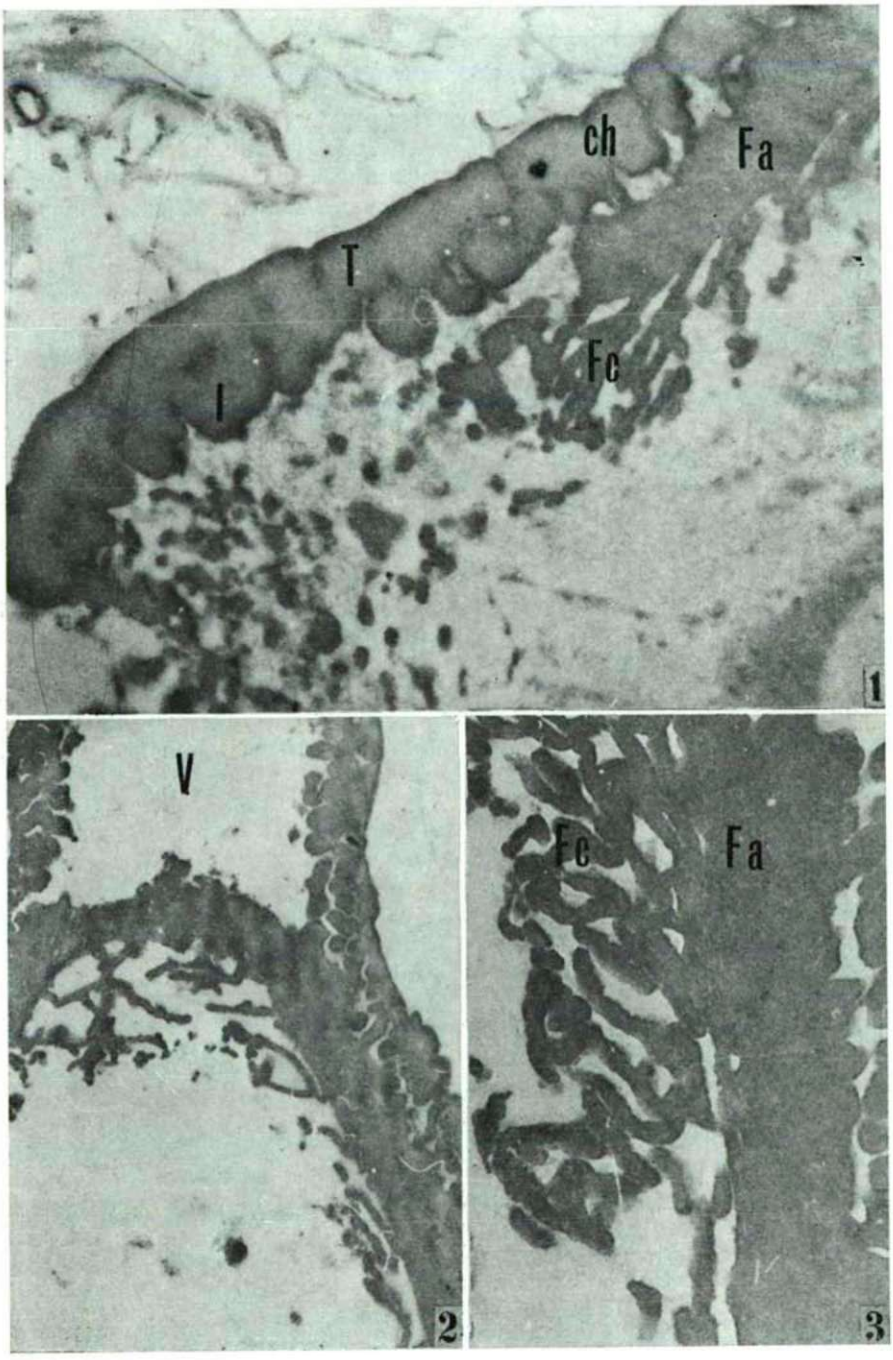


Plate II. Finer light microscopic structure of the germinal aperture of pollen of *Elaeagnus angustifolia* L. in optical section resp. top-view. x5000.

Plate III. Transmission electron microscopic structure of the pollen grain from *Elaeagnus angustifolia* L.



1. Ultrastructure of pollen grain from non-acetolyzed bud. Germinal region. x25 000.
2. Ultrastructure of pollen grain from acetolyzed flower. Germinal region. x10 000.
3. Ultrastructure of the foot layer of pollen grain from acetolyzed bud. Germinal region. x25000.

T = tectum, I = infratectum, F = foot layer, Fa = compact foot layer, Fc = foot layer with lamellar ultrastructure, ch = channels, V = vestibulum.

2. Acetolyzation generally caused an approx 11.36% increase of measurements in the majority of pollen grains.

3. During measurements in October and December, the pollen grains were maximally equal in size, though the under and upper values of measurement for pollen grains increased somewhat.

2. Transmission electron microscopic (TEM) results

Since the ultrastructural features of pollen grains from buds resp. open flowers prepared with acetolyzation resp. without it were identical, they will be discussed here together. Extragerminal exine. — Tectate, perforated with channels. Of the layers of ectexine, infratectum is the most narrow one. It is made up of narrow columns. The tectum and that part of the foot layer which is near the infratectum are µneven. Of the layers of the ectexine the foot layer is the thickest, there is no endexine.

Germinal exine. — Tectum slightly perforated in the germinal region, as in the extragerminal part (Plate III, 1). Vestibulum marked (Plate III, 1, 2), before the endoaperture the foot layer is broken up, resp. becomes lamellar (Fc). In the vestibulum, the infratectum is not columellar, it is ellipsoid, globular or irregular in shape. There are tiny granules under the exoaperture (colpus) on the pollen grains from non-acetolyzed buds (Plate III, 1). In the germinal region, the lamellar ultrastructure of the inner part of the foot layer is noticeable (Plate III, 2, 3). Its endexine cannot be observed in the germinal region.

3. Scanning electron microscopic (SEM) results

Degree of maturity investigated and acetolysis did not affect essentially the superficial ultrastructure of pollen grains. There was only one difference that on the surfaces of non-acetolyzed pollen grains, spherical, most probably pollenkitt granules occurred (Plate IV, 1, 2, 4), which were particularly frequent on the pollen grains from closed buds. It is seen that the colpi are undulated at the margins, the about 5 µm wide zone near them is slightly sculptured. Ornamentation fine, measuring generally 1–3 µm. Its character is rugulate, occasionally corrugate.

Discussion

1. Complex investigation did not reveal noteworthy differences between pollen grains from closed buds and open flowers of *Elaeagnus angustifolia* L.

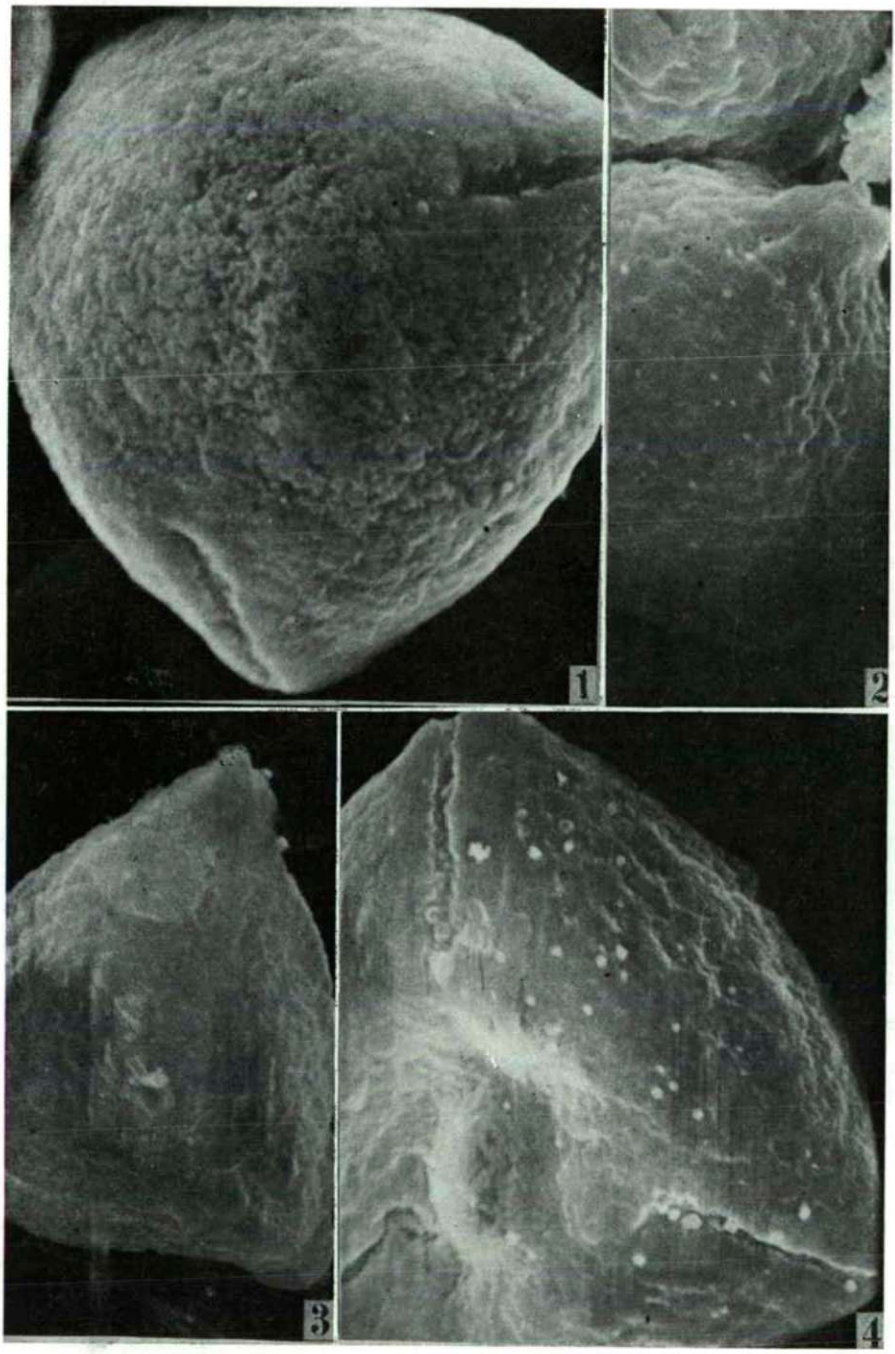
2. Only small time-dependent changes could be observed in pollen grains embedded into 39.6% hydrated glycerin-jelly. On the other hand, acetolysis considerably changed the measurements of pollen grains.

3. Of the qualitative results of light microscopic studies, the occasional occurrence of plicae, resp. pseudoplicae forms should be stressed. (The latter conception is used when two plicae occur on one surface and a third on the other one.) This is

Plate IV. Scanning electron microscopic (SEM) picture of pollen grains of *Elaeagnus angustifolia* L.

1. Non-acetolyzed pollen from flower. x2000.
2. Non-acetolyzed pollen from bud. x1000.
3. Acetolyzed pollen from flower. x1000.
4. Non-acetolyzed pollen from bud. x2000.

Plare IV



characteristic of one of the groups of Normapolles. A form-genus value has also been ascribed to this character. It should be emphasized that with fossil forms this question should be also considered with criticism, and that besides the tetraexitus forms, plicate specimens can also occur beside the "normal" ones. *Plicapollis pseudoexcelsus* is a good example for the opposite case. In a considerable proportion of its specimens plicae do not or only seldom occur.

4. Each of the complex methods, but principally the TEM one suggests that the pollen grains of *Elaeagnus angustifolia* L. may be regarded the morphological analogues of *Complexiopollis* W. Kr. 1959 em. Tschudy 1973, without supposing direct botanical relationship between the two.

Recent results gave grounds for the revision of the systematics of fossil pollen grains of Brevaxones (Kedves, 1981). At present it is the following:

1. Probrevaxones
2. Normapolles
2.1. Pronormapolles
2.2. Eunormapolles
2.3. Paranormapolles
3. Postnormapolles.

Recent analogies for Probrevaxones taxons are not known. There is only an analogue recent type for the classic form-genus of the ancient Normapolles (Pronormapolles). It is interesting that the light microscopic structure, but mainly the electron microscopic one of this group differs basically from Eunormapolles, Amentiflorae taxons, implying that there is a fairly sharp difference between the two stages of development. The establishing of transitional, connecting types is the task of further investigations.

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EFFECT OF SHORT LIGHT-DARK CYCLES ON THE CHLOROPHYLL AND CAROTENOID CONTENT OF MAIZE AND TOMATOES

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Abstract

We have studied changes in the pigment content induced by 30 to 15 and 15 to 7.5 min. light-dark cycles (LDC) on 5-week maize and 6-week tomato plants, raised in a phytotron.

In the short LDCs, primarily the pigments of the Chl a/b protein complex are damaged:

— The quantity of chlorophylls decreases, particularly the decomposition of Chl-b is considerable, thus the Chl a/b ratio increases.

— From among carotenoids, the decrease in neoxanthin and lutein mostly indicates the destructive effect of short rhythms. Antheraxanthin (lutein-epoxide perhaps) accumulates in the short rhythms.

Apart from the general tendencies of the change in pigments the following facts are obvious:

— The different sensitivity of the maize Pioneer lines 523 and 165, resp. of the two tomato species to the LDC.

— The much higher ratio of the decrease in violaxanthin in corn line 523, in the first two minutes of lighting, as compared with the dark control.

We suppose that the destructive effect of the short rhythm originates from the proton-efflux, induced by the frequent darkness, damaging primarily the plants, in which the proto-gradient, necessary for forming ATP, under the conditions of the given light intensity, quickly develops.

Introduction

For the development of more than one plant, the short light-dark cycles (alternating between 1–30 min.) are unfavourable. It was first observed by GARNER and ALLARD (1931) that in the minute rhythms plants became etiolated, leaves paler, resp. yellowish or greyish green. The tissues of the leaf necrotized with the occurance of so-called necrotic spots. In these cycles, more than one plant fully perished.

GARNER and ALLARD have not measured the change of chlorophylls but, even thus, they established correctly: "Destruction of chlorophyll seems to be an important feature in the unfavourable effect of these particular alternation of light and darkness".

Later on, the chlorophyll destruction, taking place as a result of the unfavourable LDC, was also observed by a number of authors: WITHROW and WITHROW (1949), HIGKIN and HANSON (1954), BONDE (1955), HILLMANN (1956), BONDE (1955) stated, for instance, that in the light green leaves of the *Xanthium*, developed in 5 to 15 min. rhythms, chlorophyll is only a quarter of the 12-hour control.

In these investigations, the single chlorophylls were not separated and carotenoids were not measured.

It is known from the literature and our investigations that, with the fine analysis of pigments, several informations may be obtained about the chloroplast:

— The chlorophyll a/b ration is — according to REYSS and BOURDU (1976), NIR and PEACE (1973), MARÓTI (1976a) and others — in connection with the degree of thylakoid aggregation. The chlorophyll a/b and carotene (chlorophyll-b ratios show, in addition — according to BOARDMAN and ANDERSON (1964), GROSS *et al.* (1966), BRIANTAIS (1968), MARÓTI (1976b), TUBA (1981) and others — the relative quantity of photosystems I and II, resp. of the light-harvesting chlorophyll a/b-protein complex (LHC).
— The violaxanthin cycle may give information on the pH state of the stroma and grana loculus — HAGER (1969).
— According to SIEFERMAN and YAMAMOTO (1975): "The xanthophyll cycle might be part of a regulatory system for photosynthesis that functions by influencing membrane properties."

In our paper we are showing the changes in pigment, induced as a result of LDC. On the basis of results, we attempt to show the destructive effect of the unfavourable LDC rhythms.

Materials and Methods

For the experiments we used three corns — *Zea mays* L.: 165 and 523 Pioneer* inbred corn lines, resp. 3901 hybrids — and two tomatoes — *Lycopersicon esculentum* MILL.: cv. "Kecskeméti merevszárú" (Km) and cv. "Kecskeméti 3" (K3).

Plants were raised, as described before — (MARÓTI and PATAKY 1981) — in a phytotron, at 32 W/m² light intensity and 20 ± 1 °C temperature.

The identical daily time of illumination (16 hrs) was divided into light-dark cycles (LDC) of 16–8 hours; 30–15 and 15–7.5 minutes. The rhythms of 30–15 and 15–7.5 minutes were called of short LDC, those of 16–8 hours LDC of long daytime (control).

For determining the pigment content of the leaf of maize, we used in experiment I the second and third leaves of the 4-week plants and in experiment II the fourth leaf of a 5-week maize.

The tomatoes were during the experiment 6-week old, the pigments were extracted from the third leaf.

For measuring the pigment content, the leaves were collected immediately before the experiment at 7 in the morning, before switching on the light of the control rhythm. Each ex periment was repeated four times.

For studying the violaxanthin cycle, we collected the control 3rd and 4th LDC leaves of the 5-week old maize (the dark was followed by an hour's illumination). With the leaves colleted in the dark resp. in the light, the experiment was repeated five times. No difference was found between the leaves collected in dark and light in the violaxanthin quantity.

The disks taken from both sides of the main vessels of leaves (6–8 mm diameter) were divided into four homogeneous groups (about 1 g). One of them was a "dark" control, the others were illuminated with a strong light 900 W m^{-2} for 2, 4 and 12 minutes.

The pieces of leaves were rubbed (in a weak light) in a cold friction mortar, in the presence of a little $CaCO_3$ and sand. Pigments were extracted with acetone and petroleum ether — as published before (MARÓTI and GABNAI, 1972). Chlorophylls and carotenoids were separated with thin-layer chromatography, then measured in acetone and ethanol, respectively. The extinction coefficients, published by HAGER and MEYER-BERTENRATH (1966) were used for determination pigment quantities.

* We got the kernels of corn from Dr. J. NÉMETH (Cereal Research Institute, Szeged).

Results

1. The effect of 30–15 and 15–7.5 min. LDC on the pigment content of corn- and tomato-leaves

The formation of the pigment content is determined by the general light and dark reactions, and the structure of green pigments due to the kind of plant and the structure of chloroplast. On the basis of the change in the pigment quantity, corn line 523, then hybrid 3901 were the most sensitive, and corn line 165 the most resistant to the short light treatments (Table 1). Both tomatoes responded to 30–15 and 15–7.5 LDC with a different degree of sensitivity. At Km, the degree of the decrease in pigment is particular (Table 2).

In the effect of the short LDC, there are certain general tendencies, as well, which are only modified in a small degree by the different degrees of stability provided by the peculiarities of the "kind". These are the following:

— As a result of the short LDC, the quantity of chlorophylls decreases, particularly the decomposition of chlorophyll-b is considerable, thus the ratio of chlorophyll a/b increases (Table 1, 2).
— From among carotenoides: the destructive effect of the short rhythms is most strikingly indicated by the decrease in neoxanthin and lutein.
— Anteraxanthin accumulates in short rhythms, its de-epoxidation, resp. epoxidation is inhibited.
— Corn. leaves 2 and 3 (Table 1, experiment I) are a little more sensitive to the short LDC than leaf 4 (experiment II).
— The characteristic of the "kind" manifested itself the most in the 30–15 min. LDC, because the 15–7.5 min. LDC seems to be unambiguously harmful (Table 1).

The general tendencies of the change in pigment being given, the different sensitivity of corn lines 523 and 165, resp. of the two tomato kinds, and their different responses to the short LDC are obvious (Cf. Table 2).

This difference manifests itself in the following:

— The chlorophyll content of corn line 523 in the 15–7.5 min. LDC considerably decreases, and that of line 165 hardly changes.
— The xanthophyll cycle of corn line 165 in the 15–7.5 min. LDC is strongly inhibited.
— The 30–15 min. LDC stimulates the development of corn leaf (shoot) 523 and inhibits that of 165. Thus, the decrease of chlorophylls in 523 originates partly from the growth of the leaf, and the slight increase in chlorophyll of 165 derives from the inhibited development of the leaf (in 1 g leaf there are more cells, more chloroplast), (MARÓTI and PATAKY, 1981).
— In Km tomatoes, chlorophyll-a, chlorophyll-b, lutein and violaxanthin decompose more considerably than in Kind K_3 (Table 2). It is remarkable that the considerable differences in the chlorophyll-a, chlorophyll-b, lutein and neoxanthin quatities (16–8 min. LDC) of the two tomatoes entirely disappear as a result of 15–7.5 min. LDC (Table 2).

2. The manifestation of the "kind pecularities in the violaxanthin cycle

The different sensitivity of corn lines 523 and 165 to LDC, as well as the considerable difference in pigment destructions suggested some difference in the kinetics

of deepoxidation, resp. epoxidation in leaves raised in the control 16–8 hrs LDC, as well.

According to the facts, described in the methodical part, the transformation of violaxanthin, induced by a strong light (900 W/m²), was measured in the leaves, collected from 16–8 hrs LDC (Fig. 1). This experiment with the third and fourth leaves was repeated five times, and the result was in every case that the percentile decrease of violaxanthin, as compared with the dark control, was initially much higher in corn line 523 than that of line 165. After being illuminated for 12 minutes, the percentile transformation of violaxanthin was approximately identical in the two lines (55–57 p.c.) (Fig. 1).

After the illuminations for 4, resp. 12 minutes, in leaf-disks, held in the dark for 30 minutes, the epoxidation was faster in maize 165 (Fig. 2).

Table 1. The effect of the dark-light periods on the pigment content of the corn-leaf. In experiment I, the 2nd and 3rd leaves of 4-week plants were used for the pigment analysis. In experiment II, the 4th leaf of the 5-week plants was used. At identical daily illumination, in the ligh-dark cycles (LDC), light intensity was 32 Wm^{-2}. One pigment datum is the mean of four measurements. Chl-a = chlorophyll-a; Chl-b = chlorophyll-b; Car = β-carotene; Lut = lutein + zeaxanthin; Ant = antheraxanthin + lutein-epoxide; Viol = Violaxanthin; Neo = Neoxanthin.

Corn lines	Light-treatment	mg pigment/100 g fresh weight							
		Chl-a	Chl-b	a/b	Car	Lut	Ant	Viol	Neo
Experiment I									
P 165	16–8 hrs LDC	176	44,2	3.9	13.2	17.4	1.2	8.7	5.1
	30–15 min. LDC	132	31.9	4.1	9.1	14.7	1.8	7.6	3.7
	15–7.5 min. LDC	151	35.9	4.2	9.5	14.6	1.6	8.6	4.1
P 523	16–8 hrs LDC	138	33.5	4.1	9.8	11.6	0.8	7.1	3.5
	30–15 min. LDC	92	20.6	4.4	6.7	10.2	1.7	4.4	2.7
	15–7.5 min. LDC	90	19.5	4.6	6.0	9.6	1.8	4.3	2.7
P 3901	16–8 hrs LDC	134	33.6	3.9	11.3	13.3	0.9	7.2	3.4
	30–15 min. LDC	100	21.8	4.5	7.6	10.8	2.0	4.9	2.7
	15–7.5 min. LDC	115	21.2	5.4	8.1	12.0	1.8	5.4	2.4
Experiment II									
P 165	16–8 hrs LDC	133	30.3	4.3	10.9	13.8	1.6	7.9	4.5
	30–15 min. LDC	142	33.0	4.3	11.0	17.3	2.5	8.2	3.9
	15–7.5 min. LDC	140	32.6	4.3	11.4	15.2	2.2	8.5	4.0
P 523	16–8 hrs LDC	122	29.0	4.2	8.8	11.2	0.9	5.8	3.5
	30–15 min. LDC	103	22.1	4.6	7.9	11.0	1.8	5.4	2.7
	15–7.5 min. LDC	80	17.3	4.6	5.5	9.7	1.7	5.2	2.6
P 3901	16–8 hrs LDC	115	25.0	4.6	8.9	12.6	1.0	4.9	3.3
	30–15 min. LDC	130	28.7	4.5	9.2	14.0	1.6	7.3	3.4
	15–7.5 min. LDC	107	22.8	4.7	8.7	12.2	1.8	6.2	2.5

Table 2. The effect of the 30–15 and 15–7.5 min. LDC on the pigment content of the 3rd leaf of 6-week tomatoes. The experimental conditions, abbreviations are to be found in Table 1.

Toma-toes	Light-treatment	mg pigment/100 g fresh weight							
		Chl-a	Chl-b	a/b	Car	Lut	Ant	Viol	Neo
K_3	16–8 hrs LDC	90	28.6	3.1	6.8	9.8	1.0	3.9	3.0
	30–15 min. LDC	60	17.2	3.5	5.1	7.5	1.5	3.3	2.7
	15–7.5 min. LDC	58	14.8	3.9	4.4	6.7	1.3	3.6	1.7
Km	16–8 hrs LDC	109	34.9	3.1	7.7	12.0	1.0	3.8	3.9
	30–15 min. LDC	73	21.1	3.4	6.2	8.7	1.3	3.0	2.5
	15–7.5 min LDC	53	13.8	3.8	6.0	6.9	1.8	2.7	1.7

Discussion of results

1. Is there a pigment destruction or are formed etiolated leaves in LDC of 30–15 and 15–7.5 minutes?

It was demonstrated by more than one research worker that in case of an alternating light-dark cycle, where 1 msec flashlight was followed by 15 min. darkness (Strasser and Sironval 1972, Strasser and Butler 1976) — or a light of 2 minutes by 98 minutes darkness (AKOYUNOGLOU, 1977; AKOYUNOGLOU and ARGYROUDI; AKOYUNOGLOU, 1978) — the leaves remain etiolated.

In these leaves, chloroplast is unable to develop oxygen and does not contain grana. In the so-called primary thylakoid, the synthesis of pigments, lipids and proteins, as well as their integration into functional units are inhibited. The possibility is raised that in the short rhythms, described here, leaves are etiolated, resp. some similar inhibitions of synthesis take place.

Despite the pale green colour of leaves, we do not share this opinion. According to our supposition, the decrease of the pigment content primarily originates not from the inhibition of synthesis but from the destructive effect of the 30–15 and 15–7.5 min. LDC. This is shown by the following:

— The light quantity in the 30–15 and 15–7.5 min. LDC corresponds to the considerable illumination of daily 16 hrs. The plants (beans, tomatoes) flower and may ripen fruit, in spite of their pale green colour.
— The chlorophyll a/b ratio is not high.
— We (MARÓTI and MARÁCZI, an unpublished datum) have observed that pigments also decompose if tomatoes having raised under natural conditions get a short LDC treatment.

2. In the short rhythms, the pigments of the chlorophyll a/b protein complex II are damaged

We (MARÓTI and GÁBOR, 1976; MARÓTI, 1976) demonstrated that the Lut, Neo and chlorophyll-b content of the spongy parenchyma chloroplasts, containing several grana, is more than that of the palisade chloroplasts, rich in stroma lamellae.

On the basis of literary data, we connected the high Lut, Neo and Chlorophyll-b content with the increased activity of photosystem II.

From the photochemically active photosystem II, the light-harvesting Chlorophyll a/b-protein complex (LHC) may be separated (THORNBER, 1975). The pigment composition of this complex is known from the work of Siefermann–Harms (1980) (Table 3).

Table 3. Pigment content of light-harvesting chlorophyll a/b-protein

Species	Pigment/100 Chl-a				
	Chl-b	Car	Lut	Viol	Neo
Phaesolus vulgaris	62.5	0.6	30.7	2.0	10.0
Spinacia oleracea	77.0	1.3	31.0	8.9	13.1

It is to be seen from the Table that in LHC the lutein, neoxanthin and Chl-b contents are high and carotene is in traces. It is known (SIEFERMANN–HARMS, 1980) as well, that in phtosystems I and II, 13, resp. 12.3 carotenes fall to 100 Chl-a molecules. We suppose that the quantity of the pigmenta of the Chl a/b protein complex II is proportional to the content of the complex protein. On the basis of these, the quantity of LHC can be established from the relative difference of the carotene and lutein quantity, from the neoxanthin and Chl-b contents. Among corn lines, the LHC of highest number or largest size belongs to P 165; this is followed by P 3901 and the smallest belongs to P 523. From among tomatoes, line Km has a higher LHC content than line K_3.

The fundamental effect of 30–15 and 15–7.5 min. LDC is to damage LHC. (Tables 1, 2).

As a consequence of shorter LDC the decomposition of Lut and Neo comes to a halt at the sensitive kinds, as well. In tomatoes, the different contents of Lut and Neo will be identical in 15–7.5 min. LDC (Table 2).

These results suggest that not the complete LHC is damaged by the short LDC only one of its components, on the other hand this component's different size depends on the "kind" property.

Another characteristic of the short rhythms is the increasement in the quantity of antheraxanthin. As antheraxanthin and lutein-epoxide are not separated on our thin-layer slide, probably it is not the de-epoxidation of antheraxanthin that is inhibited but just the epoxidation of lutein takes place.

From among corn lines, No. 523, with the least LHC, and from among tomatoes, Km, with the most LHC, were mostly damaged by the 15–7.5 min. LDC. Clearly the destructive effect of short rhythms is not directly connected with the quantity of LHC.

3. What causes the unfavourable effect of the short rhythms?

Since MITCHELL's chemiosmotic theory of ATP synthesis (1961, 1977), a number of researches — HIND and JAGENDORF, 1963; CROFTS *et al.*' 1972; PICK *et al.*, 1973 and others — demonstrated that in the thylakoid loculi (from one side of the

membrane to the other (light-induced proton-uptake and — as a result of darkness — from the intrathylakoid space — proton emission take place. We suppose that, in the 30–15 and 15–7.5 min. LDC, owing to the frequently repeated dark period:

— The H^+/Mg^{2+} exchange between the partition and loculus is partial.
— The Mg^{2+} bond between light-harvesting Chl-a/b-protein complexes, the close adhesion of grana thylakoids — MURAKAMI and PACKER (1970), BARBER (1976), BARBER and CROW (1979) — become loose resp. defective, owing to the repeated proton efflux.
— Owing to the defective adhesion, the surface of LHC, resp. Lut, Neo and Chl-b, on it, become and start decomposing.

Our suppositions are supported by the electron-microscopical photographs, as well, being in preparation, but we need a further many-sided investigation in order to decide the question.

4. The violaxanthin cycle is the indirect indicator of proton-transport

According to HAGER (1969), the de-epoxidation (transforming into zeaxanthin through anteraxanthin) of violaxanthin in the isolated chloroplast can be elicited by the low pH both of the light and medium. HAGER supposed that the de-epoxidase enzyme is to be found in the loculus and is activated by the low pH.

It was demonstrated by MARÓTI and SZAJKÓ (1972) that at the light-induced transformation of violaxanthin in leaf-disks:

— Violaxanthin occurs in chloroplasts in strongly and weakly bonded forms.
— In de-epoxidation, primarily the weakly bonded violaxanthin takes part.

Later on, SIEFERMANN and YAMAMOTO (1974, 1975) observed more exactly on isolated lettuce chloroplasts that:

— Maximum two-thirds (67 p.c.) of the entire violaxanthin quantity can be transformed and only this participates in de-epoxidation.
— The violaxanthin quantity, able to be de-epoxidized, changes depending on light intensity.
— They have supposed that the violaxanthin which is able to be de-epoxidized is to be found in the loculus (mainly as a "free pigment"), and the one-third of violaxanthin that is unable to be transformed is to be found at the outer surface of the membrane (mainly as protein-bound) (SIEFERMANN–HARMS, 1980).

It follows from the above mentioned that — at the given light intensity — the activity of de-epoxidase (the quantity of violaxanthin transformed within the time unit (may be the endogenous indicator of the light-induced acidification of the loculus

In our experiments it has been observed that:

— As a result of high light-intensity (900 W/m^2), the light-induced decrease of. violaxanthin (the percentage of dark control), in corn line 523, is three times faster in the first two minutes than in 165 (Fig. 1).
— As a result of the 15–7.5 min. LDC, in corn line 523, violaxanthin considerably decreases, and in corn line 165, it increases (Table 1).
— The mesophyll chloroplasts of line 523 shrink in LDC and those of 165 swell. At the same time, line 523 produces in this rhythm the most, and line 165 the least dry material (Maróti and Pataky, 1981).

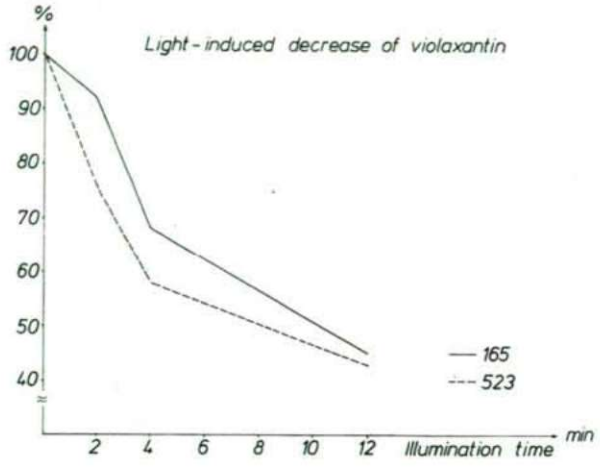

Fig. 1. Light-induced decrease of violaxanthin (percentage of dark control) in corn lines 165 and 523. Light intensity is 900 W/m². The experiment was performed with disks of 6 mm diameter taken from the third and fourth leaves of 5-week maize, on wet filter-paper.

Consequently in the chloroplasts of line 523, the proton gradient, necessary for ATP, is earlier formed (even at light intensity 32 W m²) than in corn line 165. The 15 min. illumination of chloroplasts 523 is satisfactory for the de-epoxidation of a violaxanthin; the same illumination of corn line 165, however, is insufficient. This may be one of the causes of the opposed change of the violaxanthin content.

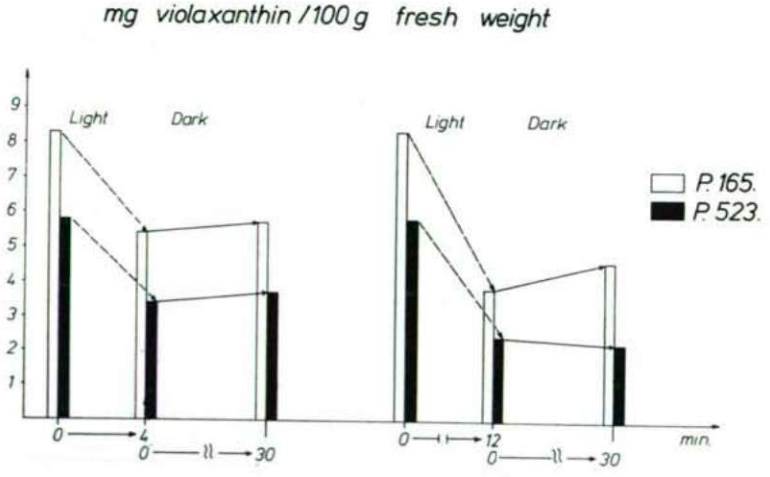

Fig. 2. De-epoxidation of violaxanthin in light and epoxidation of zeaxanthin in the dark. The time of illumination was 4, resp. 12 minutes, the dark period 30 minutes. The experimental conditions are identical with those of Figure 1.

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EFFECT OF ALTERNATING LIGHT-DARK CYCLES ON THE SIZE OF MAIZE CHLOROPLASTS AND ON THE ACCUMULATION OF DRY MATTER

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Abstract

We have studied the effect of the short light-dark cycles (LDC) of 30–15 and 15–7.5 min. on the size of chloroplasts and the accumulation of dry matter, on 5-week corn lines (Pioneer 165 and 523) and a hybrid (No. 3901), raised in a phytotron (32 Wm^{-2}).

Between the shrinkage of mesophyll-chloroplasts and the accumulation of dry matter, a positive connection was found in the 30–15 min. LDC.

The two corn lines (165 and 523) responded to the 30–15 min. LDC contradictorily. The size of bundle sheath chloroplasts is in a positive correlation with the dry-matter production, its volume is determined to a great extent by the size and number of grains of starch contained in it.

The 15–7.5 min. LDC considerably decreased the size of bundle sheath chloroplasts and reduced the quantity of dry matter.

We suppose that in the short LDC the frequent dark-induced proton efflux disturbs the normal formation of grana and, on the other hand, inhibits the formation of the necessary pH difference on the two sides of the thylakoid membrane.

Introduction

According to GARNER and ALLARD (1931), ALLARD and GARNER (1941), BONDE (1955), TUKEY and KETELLAPPER (1963), the equal light-dark cycles of 5 sec. each, 1, 4 and 12 hours each, are favourable for the accumulation of dry matter.

The optimum of the not equal light-dark cycles is shown, according to KETELLAPPER (1965), by the ratio between the length of light periods and that of the full cycle, the value of the so-called photofraction. The considerable inhibition of growing by the short (1–30 min.), approximately equal light-dark rhythms, and their strong reducing effect on the accumulation of dry matter were demonstrated by many researchers (GARNER and ALLARD, 1931; PORTSMOUTH, 1937; BONDE, 1955; FOGG, 1968; RAJAN et al., 1971; MARÓTI et al. 1980).

About the effects of light-dark cycles at cell level, only few data are known.

The light-induced *in vivo* shrinkage of chloroplast was demonstrated by some researchers thus KUSHIDA et al. (1964), HILGENHEGER and MENKE (1965), MAKAROV et al. (1976), NOBEL (1967), ZURZYCKI (1974). Taking the volume of chloroplast held in the dark as 100 per cent, the decrease of volume changed between 15 and 30 per cent. HELDT et al. (1973) could not observe any light-induced change in the size in isolated chloroplasts.

According to ZURZYCKI and METZHER (1977): "the shape of chloroplasts depends on two factors: the rigidity of the internal structures and mechanical effects of the surrounding cytoplasm". That's why we studied the effect of the short light-dark cycles on the size of chloroplasts. On the other hand, it was demonstrated by

MURAKAMI and PACKER (1970), HIND et al. (1974), KRAUSE (1977), DUNIC et al. (1979) that the reduction of the size of chloroplast is in connection with light-induced ion (H^+, Mg^+, K^+) exchanges, the loss of water, attenuation of the membrane The attenuation of membranes orginates from the energizing effect of the light-induced proton gradient.

After all, the degree of the shrinkage of chloroplasts depends on the intensity of the photosynthetical light reaction. Thus, it is to be supposed that from the changes in the size of chloroplasts we can draw conclusions concerning the organic matter production, as well.

According to MUSTÁRDY et al. (1976, 1981), as a result of the light and Na^+ ions, the membranes of stroma taper off in a much lower degree than the membranes of grana. The considerable attenuation of the normal membranes of grana takes place by the participation of the light-harvesting Chl a/b protein complex (LHC). The change in the size of chloroplasts depends, therefore, primarily on the number and size of grana and this may be interesting at comparing the "kind" properties. Of the nearly equal light-dark cycles induced changes in chloroplast there is only known a little information, obtained by an electron microscope (e.g. REYSS et al., 1970; HIRSCHAUER et al., 1971).

The 30–15 min. light-dark rhythms induce considerable histological and morphological changes in beans and mustard (MARÓTI et al. 1980). In our experiments in the phytotron, we have, therefore, looked for an answer to:

— What kind of effect is exerted by the 30–15 and 15–7.5 min. LDC on the size of chloroplasts and if the change in the *in vivo* sections can be measured by a light microscope?

— Is there any difference in the change of size of mesophyll and bundle sheath chloroplasts?

— What kind of conclusion may be drawn from the size of chloroplast concerning photosynthesis, the accumulation of organic matter?

Do the single corn lines respond to light treatment equally?

Materials and Methods

For the experiment, *Zea mays* L: Pioneer inbred corn lines 165 and 523, resp. Pioneer hybrid 3901 were used.* The plants were raised in a phytotron (HORVÁTH, 1972), with 400–700 nm wave-length interval, under light-tubes F_{29}, at 20 ± 1 °C daily temperature and 50–70 per cent relative moisture-content.

At identical daily illumination, light treatments were as follows:

(a) Light-dark rhythms (LDC) of identical intensity: 16–8 hrs; 30–15 resp. 15–7.5 min. light-dark periods. Light intersity: 32 Wm^{-2}.

(b) Light-light rhythm (LLC) of changing intensity: 16-8hrs. 30 min. (30.75 Wm^{-2})–15 min. (2.5 Wm^{-2}) light-light periods.

Plants were raised in a mixture of sand-pearlite of ration 1:1, with Hoagland's nutrient solution, modified by Reyss and Bourdu (1971), till their 5-week age. Water capacity of the sand-pearlite culture was daily set with distilled water to 70 per cent. In an experiment, in four climatic chambers (with four kinds of light-treatment), in 4 times 8 pots 96 plants were raised from one "kind" of plants. The experiment was repeated 3 times in different times. The size of chloroplasts was established on the basis of two experiments and the quantity of dry matter from the means of the three experiments. The plants were elaborated at 5-week old. The dry weight was established after being fixed at 105 °C and then dehydrated at 70 °C, in each organ

* Seed-corn obtained from Dr. János Németh, Cereal Research Institute, Szeged.

separately. For investigating into the size of chloroplasts, we took samples from the middle region of the fourth leaf (counted from below) of the 5-week old plants. There were collected as controls the leaves of plants raised in 16–8 hrs LDC, in experiment I after 1hr illumination (at 8 o'clock in the morning), in experiment II at the end of an 8-hour long dark period (at 7 o'clock). From the other rhythms, leaves were taken at the end of the light period. (Three leaves in each of the experiments and light treatments.) Leaves were cut by hand, resp. with an electrical (Kryomat) refrigerating microtome of type Leitz. The 20–30μ thick sections were covered with 1.5 p.c. glutar-aldehyde, 0.5 M potassium-phosphate (pH 7.3) buffer and immediately photographed with a polerizing photomicroscope Opton. The longest diameter and thickness of the chloroplast were measured from paper pictures of known magnification (N: 1969). In each experiment: one photographic datum is the mean of 60 measurements.

Results

1. The effect of the short light-dark periods on the quantity of dry matter

The effect of the 30–15 min. LDC on the investigated dry-matter accumulation of corn lines was different. This is particularly striking in lines 523 and 165 (Table 1).

In the 30–15 min. LDC, the total dry matter of corn 523 increased by 23 per cent and that of the leaf by 38 p.c., as compared with the 16–8 hrs LDC. On the other hand, at corn line 156, the dry weight, falling on each leaf, has not changed and the total dry matter decreased by 10 p.c., as compared with 16–8 hrs LDC of the control (Table 1).

The dry-matter accumulation of hybrid 3901 corn was less stimulated by the 30–15 min. LDC than that of line 523 and was less inhibited than that of line 165.

Table 1. The effect of the light-dark periods of different length on the dry-matter quantity of 5-week old corns.

At identical daily illumination, in the 16–8 hrs, 30–15 and 15–7.5 min. LDC, light intensity was 32 Wm^{-2}; in the 30–15 min. LLC of continuous illumination, light intensity changed between 30.75 Wm^{-2} and 2.5 Wm^{-2} (30–15 min.). The following are the means of 36 plants in three experiments.
("Other" = leaf sheath, leaf and stalk primordia.)

Corns	Light treatment	Dry weight mg/plant			
		root	leaf	other	total
P 165	16–8 hrs LDC	468.9	280.6	111.9	861.4
	30–15 min. LDC	415.7	273.5	88.9	778.1
	15–7.5 min. LDC	492.2	223.5	75.3	791.0
	30–15 min. LLC	511.8	278.0	111.7	901.5
P 523	16–8 hrs LDC	472.6	222.6	97.0	792.2
	30–15 min. LDC	528.1	307.2	112.5	974.8
	15–7.5 min. LDC	419.2	206.6	75.3	701.1
	30–15 min. LLC	475.0	238.0	95.4	808.4
P 3901	16–8 hrs LDC	725.0	400.8	170.2	1296.0
	30–15 min. LDC	662.5	489.5	169.6	1321.6
	15–7.5 min.LDC	678.0	335.0	127.3	1140.3
	30–15 min. LLC	681.2	455.5	168.9	1305.6

Hybrid 3901 has outstandingly utilized the light, in spite of light intensity 32 Wm^{-2} which is low for corns. As compared with the the two lines, it gave a maximum dry-matter production in every rhythm. The 15—7.5 min. LDC considerably diminished the quantity of dry matter, both at the two lines and the hybrid. The degree of the total dry-matter decrease was the highest (12 p.c) at corn line 523 (Table 1).

The increase in the dry-matter of corn lines was favourably influenced by the continuously illuminated 30–15 min. LLC of rhythmically changing strength. As compared with the 16-8 hrs LDC, in the 30–15 min. LLC the increase in dry matter is insignificant but the difference is striking if we compare the dry-matter productions induced by the 30–15 min. LDC and the 30–15 min. LLC.

The 30–15 min. LDC increased the dry-matter quantity of corn line 523 by 23 per cent and decreased that of corn line 165 by 10 per cent. On the other hand, the 30–15 min. LLC of continuous light moderated the accumulation of the total dry matter at corn line 523 and increased it at 165, as compared with the 30–15 min. LDC.

The short rhythms of 30–15 and 15–7.5 min. caused only a slight change in the dry-matter accumulation in the organs of corn lines. The "favourable" resp. "in-favourable" effect of the short light-dark periods primarily manifests itself in the dry-matter quantity of the intensively growing leaves, leaf sheaths and stalk- and leaf-primordia.

2. The effect of alternating light-dark cycles on the size of chloroplasts

Corn lines 165 and 523 considerably differ from each other in the size of chloroplasts and in their reactions given to the 30–15 min. LDC, as well.

The mesophyll and bundle sheath chloroplasts of line 165 are larger than those of corn line 523 (Table 2).

The 30–15 min. LDC hardly changed the diameter of mesophyll chloroplasts (Mchp) of corn line 165, at the same time, however, it considerably increased their thickness. Opposite to the swelling of Mchp 165, the diameter and thickness of Mchp of corn line 523 also considerably decreased (Table 2, Fig. 1).

The full volume of bundle-sheath chloroplasts (Bchp) was considerably increased by 30–15 min. LDC in case of both corn lines (165 and 523). It is to be seen even with a light microscope that chloroplasts — with the exception of 15–7.5 min. LDC — are full of starch (Table 2, Plate I, picture 3; Plate II, pictures 2, 3; Plate IV, pictures 3, 4). At corn line 523, the dark green colour of Bchp is striking; its cells are full of chloroplasts (Plate II, picture 2).

The diamater of the Mchp-s of line 165 was a little reduced by the 15–7.5 min. LDC. It is obvious that in case of corn line 523, the change in Mchp is insignificant. At the change in Mchp-size, it can less be observed that the 15–7.5 min. LDC is unfavourable for the development of plants, for the accumulation of dry matter. The damaging effect of this rhythm is, however, shown by several facts:

— As a result of the 15–7.5 min. LDC, the size of Bchp-s strikingly decreases at both corn lines.

— In Bchp, hardly any starch can be seen.

— The Mchp-s are yellowish green, very easily burst asunder, break in to pieces.

(Table 2., Plate III, pictures 1, 2, 3.)

The diameter of Mchp-s of corn line 165 was a little reduced by the 30–15 min. LLC of continuous illumination but of rhythmically changing strength, its thickness was considerably increased by the same. The size of Mchp-s of corn line 523 was somewhat reduced in this rhythm, resp. it hardly changed (Plate IV, pictures 1, 2).

The Bchp-s are the largest in the 30–15 min. LLC, they are full of starch (Plate IV, pictures 3, 4).

The change in the thickness of Bchp-s shown an opposite tendency at the two lines. At line 165, the thickness of Bchp-s considerably increases, in contradiction to line 523, where the thickness of Bchp-s only a little changes, as compared with the control and the 30–15 min. LDC (Fig. 1).

Apart from the differences, manifestina themselves in the size of the chloroplasts of the two lines and in the tendency of chloroplasts to change their size, the tissue structure of leaves also considerably changes, as a result of treatments. In addition to phenological observations, leaf cross-sections were also measured. On the basis of some not published results, the most obvious changes are the following:

Table 2. The effect of light treatments of short rhythm on the size of chloroplast of the fourth leaf of 5-week old corn lines.

The light treatments are identical with those described in Table 1. The chloroplasts of 16–8 hrs LDC were measured in experiment I after 1 h illumination, and in experiment II at the end of an 8 hrs dark period. At the other rhythms, the chloroplasts originate from the end of the light cycle. The chloroplast diameter means the longest diameter resp. largest thickness in μ. (The data are the means of 60 measurements.)

Maize lines	Light treatment	Change in the size of chloroplasts (μ)			
		mesophyll chl. pl.		bundle sheath chl. pl.	
experiment I		diameter	thickness	diameter	thickness
P 165	16–8 hrs LDC	5.3	3.1	7.9	5.3
	30–15 min. LDC	5.3	4.4	8.5	5.6
	15–7.5 min. LDC	4.5	3.4	6.8	4.5
	30–15 min. LLC	5.0	4.1	8.6	6.1
P 523	16–8 hrs LDC	4.3	3.2	6.4	4.3
	30–15 min. LDC	3.9	3.0	6.9	4.9
	15–7.5 min. LDC	4.2	3.1	6.1	3.6
	30–15 min. LLC	4.1	3.1	7.1	4.8
experiment II					
P 165	16–8 hrs LDC	5.4	3.4	7.6	5.1
	30–15 min. LDC	5.6	4.7	8.6	5.8
	15–7.5 min. LDC	4.9	3.5	7.1	4.4
	30–15 min. LLC	5.1	4.2	8.7	6.0
P 523	16–8 hrs LDC	4.2	3.2	6.6	4.5
	30–15 min. LDC	3.8	2.8	7.3	5.3
	15–7.5 min. LDC	4.3	3.1	6.0	3.6
	30–15 min. LLC	4.2	3.0	7.2	4.6

— The thickness of cell-walls (mesophyll, epidermis) are reduced by the 15–7.5 min. LDC. The cells are "expanded". The motor cells are strikingly increased. They run to about 50 per cent of the thickness of the leaf-blade. This refers to increased water content, as well. The water quantity, falling on 1 g dry weight in the 15–7.5 min. LDC, at corn line 523, is 19 per cent and at corn line 165, 2 per cent — more in the fourth leaf, as compared with the control. The large vascular bundles are normally developed (Plate IV, picture 4; Plate V, picture 6).
— The leaves of the individuals, grown in the 30–15 min. LDC and 30–15 min. LLC, are more "massive". In case of line 523, particularly the bundle sheath cells are filled, almost fully, by chloroplasts. In case of line 165, this effect is more moderated (Plate V, pictures 3, 4, 5).
On the leaves of control individuals the changes described above cannot be observed (Plate V, pictures 1, 2; Plate I, picture 4).

Discussion

1. Dry-matter production and the length of the light-dark cycles

At establishing the optimum length of the light-dark cycles, we should take into consideration that certain plants need a different light-dark period for the generative and vegetative developments repectively. We have only investigated in our experiments the vegetative growth. Thus we consider as optimum the LDC, producing the highest dry-matter production during the time unit with identical illumination and light quantity.

According to HORVÁTH et al. (1977, 1978), MARÓTI et al. (1980), the 30–15 min. and even longer LDC may have, depending upon the peculiarity of the kind, several optimum lengths, too. On the other hand, the 15–7.5 min. LDC is unambiguously unfavourable for the accumulation of dry matter. The time of daily illumination and the quantity of energy are identical in the 16–8 hrs, 30–15 min. and 15–7.5 min. LDC. So the question rises, why the 15–7.5 min. LDC is harmful.

The quantity of dry matter depends — according to ALLARD and GARNER (1941) — primarily on the length of light periods.

The optimum length of LDC can be best illustrated — according to TUKEY et al. (1963), KETELLAPPER (1965) — by the ratio between the length of the light period and that of full cycle. If this ratio is 5/6 (e.g.: 20–4 hrs, 30–6 and 15–3 min. LDC), then a maximum dry matter can be obtained.

According to the literary data, in our LDC the length of the light period is short. HURD's investigation (1973) seems to support this, as well. He changed — at an identical daily illumination of 8 hrs (20 Wm^{-2}) light — 16 hrs darkness; 8 hrs (18 WM^{-2}) light + 8 hrs (2 Wm^{-2}) light — 8 hrs darkness — light intensity and the length of the LDC period. In photoperiod 8+8 light — 8 darkness he obtained 100 per cent more dry matter than in the 8–16 hrs LDC.

Despite the literary data, seemingly the unfavourable effect of short rhythms is only slightly connected with the relative length of the light-period, because:

— The ratio of the length of the light-period and that of the full cycle was identical in all LDC, i.e. 4/6.
— In the same 30–15 min. LDC, the total dry matter of corn line 523 is the most, and that of corn line 165 is the least, as compared with the other light treatments.

— In the plants investigated by the authors of this paper (maize, tomatoes, beans), the quantity of dry matter was strongly reduced by 15–7.5 min. LDC. This considerable inhibition of the short rhythms is surprising because the second- and the a-few-seconds-long LDC-s produce an outstanding organic matter (GARNER and ALLARD, 1931; FOGG, 1968).

According to our supposition, the unfavourable LDC-s primarily disturb the accumulation of thylakoid loculi and the formation of the difference of pH, necessary on the two sides of the membrane. The damaging factor originates from the often repeated dark periods, from the summarized proton emission (MARÓTI et. al, 1981). All these manifest themselves initially in the trouble of proton-transport, the swelling of the chloroplast, and later on, in the destructions of the membrane and the decrease in size of the chloroplast.

2. The volume of chloroplast and the production of organic matter

Our experiments confirm NOBEL's observation that the shrinkage of chloroplasts refers to an intensive photosynthetic functioning. The close coincidence of the smallest size of the investigated corn Mchp-s and the largest quantity of the total dry matter is striking in the 16–8 hrs and 30–15 min. LDC-s (Fig. 1).

There is an opposite change in the size of the Mchp-s of the two corn lines and the accumulation of dry matter in the 30–15 min. LDC. The size of Mchp 523 in this LDC is the smallest and largest dry matter production. The Mchp thickness of line 165 is strikingly the largest in the 30–15 min. LDC. At the same time, its dry

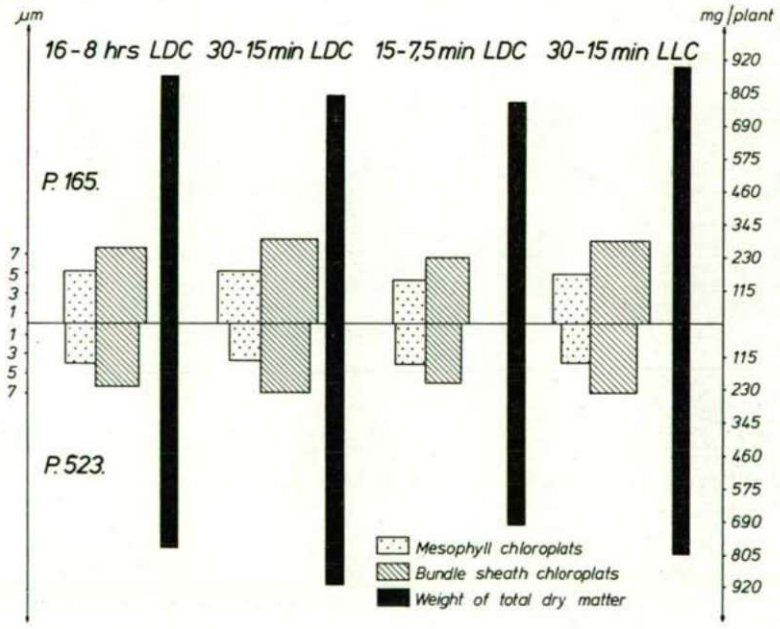
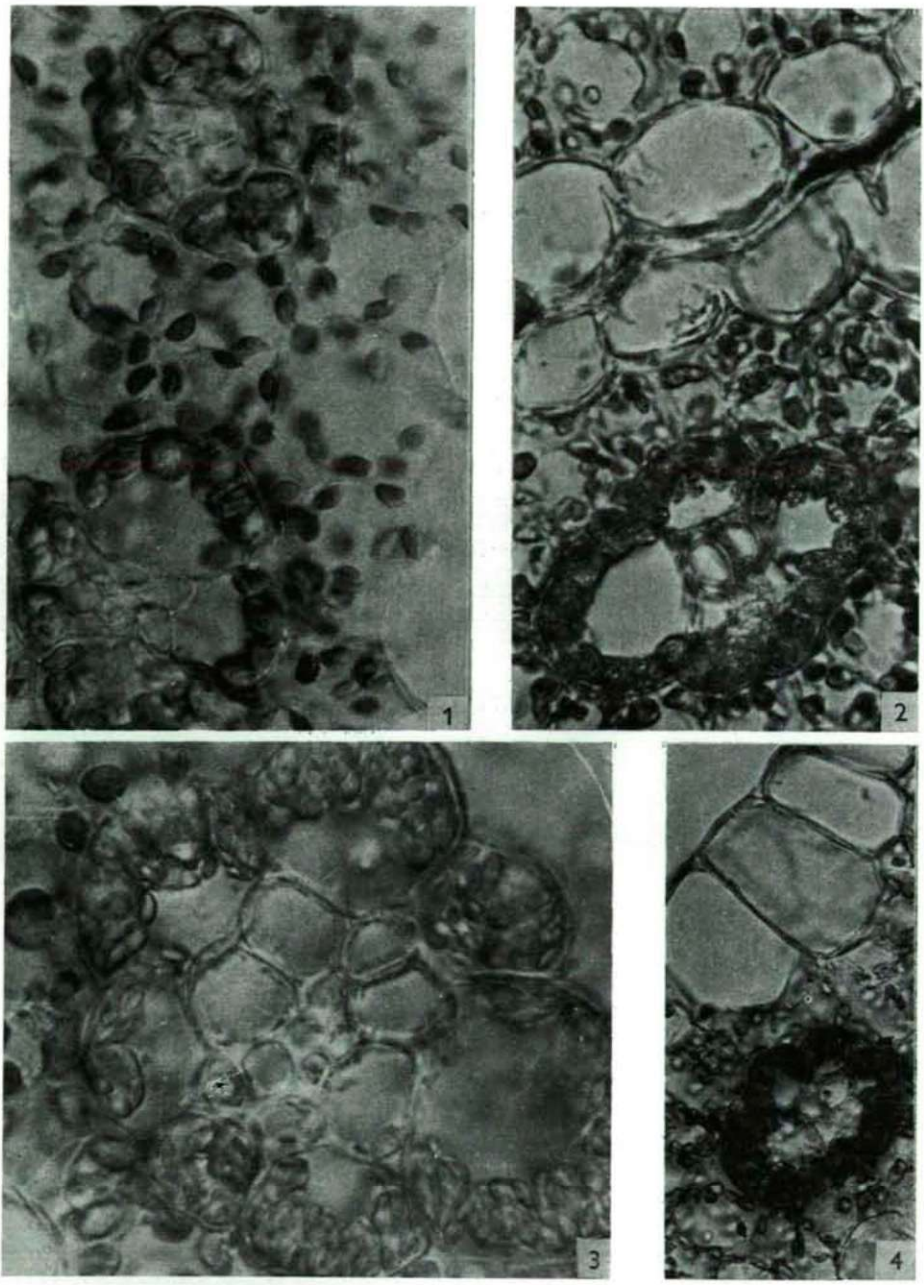


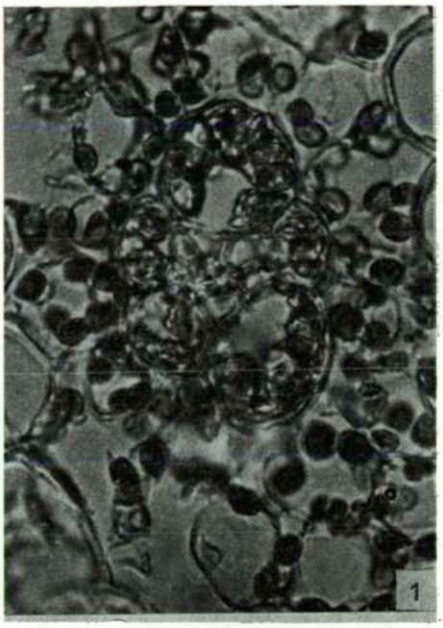
Fig. 1. Size of mesophyll and bundle sheath chloroplasts and change in the total dry weight, due to the 16–8 hrs, 30–15, 15–7.5 min. light-dark cycles (LDC) and to the 30–15 min. light-light cycle (LLC).

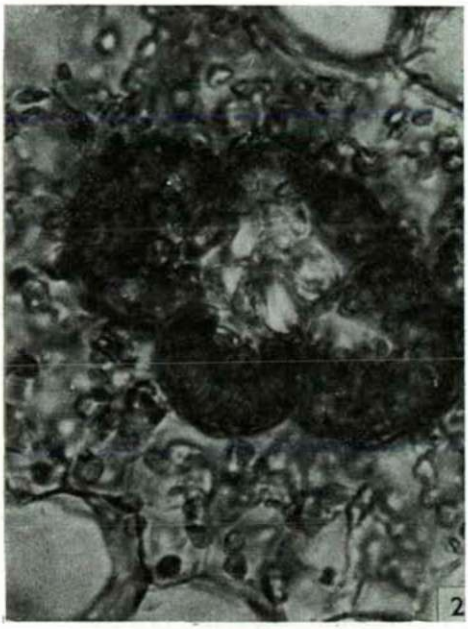
Plate I. Effect of the 16–8 hrs LDC.

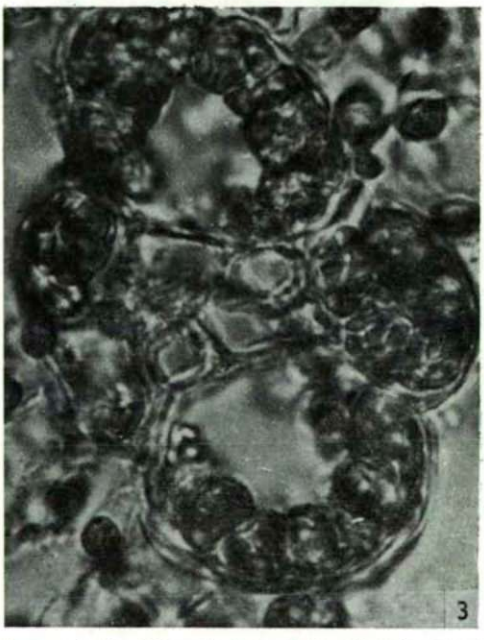


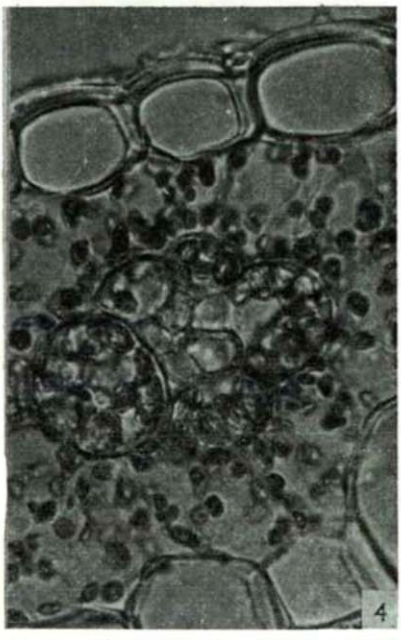
Picture 1: Pioneer line (P. 1.) 165, Picture 2: Pioneer line 523: the difference by size of mesophylls and bundle-sheath chloroplasts of identical magnification (x1000). Picture 3: Grains of starch in the bundle-sheath chloroplasts, P. 1. 165, Magnification: x1580. Picture 4: leaf cross-section, motor cells, P. 1. 165, magnification: x545.

Plate II. Effect of the 30–15 min. LDC.



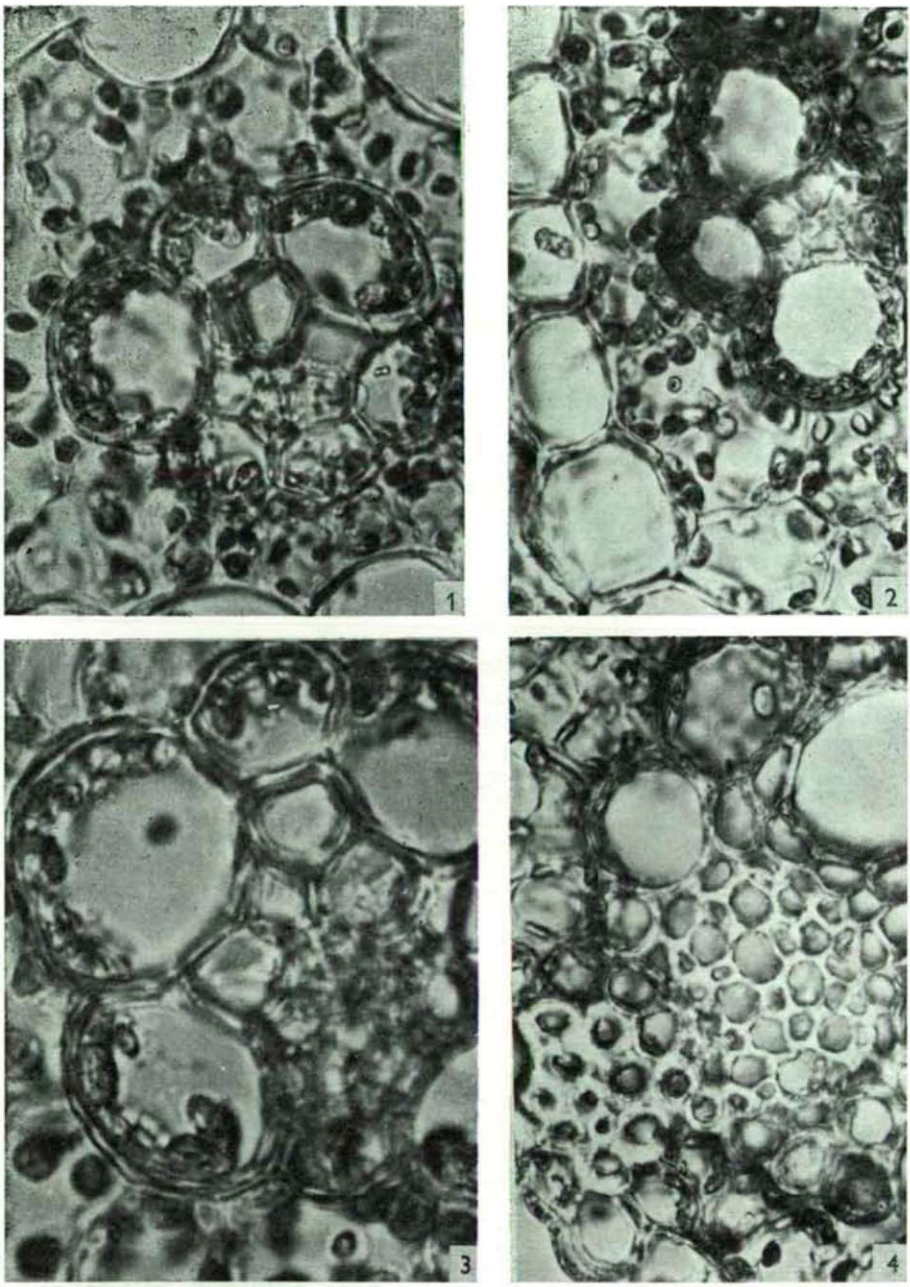






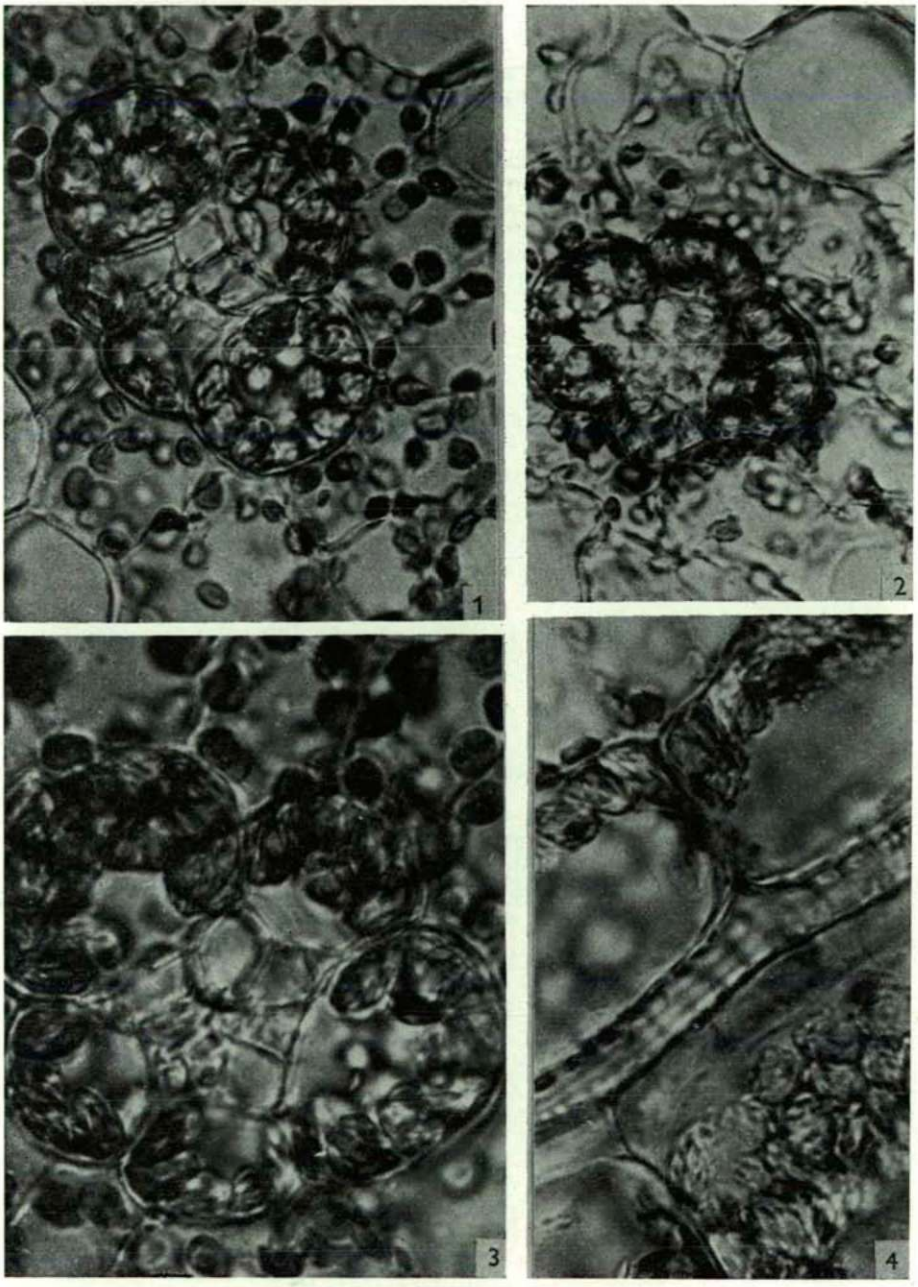
Pictures 1, 3: Mesophyll and bundle sheath chloroplasts of P. 1. 165, magnification: x1000 (picture 1), x1580 (picture 3). Picture 2: mesophyll and bundle sheath chloroplasts of P. 1. 523, magnification: x1000. Picture 4: Leaf cross-section of P. 1. 523, magnification: x810.

Plate III. Effect of the 15–7.5 min. LDC.



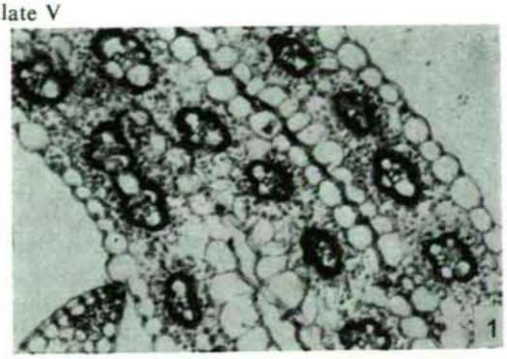
Picture 1: P. 1. 165, Picture 2: P. 1. 523: degraded mesophyll and bundle sheath chloroplasts of identical magnification (x1000). Picture 3: in the bundle sheath chloroplasts of centrifugal arrangement there can hardly be seen any grains of starch. P. 1. 165, magnification: x1580. Picture 4: the sclerenchyma-, phloem-xylem-part- and bundle sheath-cells of the cross-section of large veins. P. 1. 523, magnification: x1000.

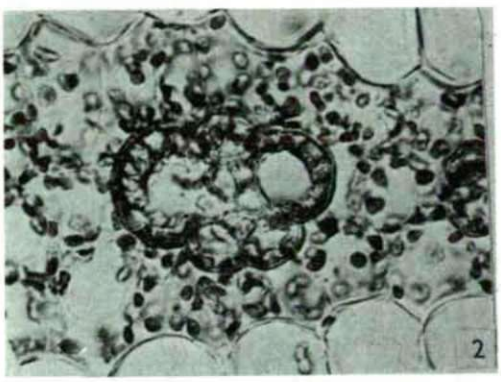
Plate IV. Effect of the 30–15 min. LLC.

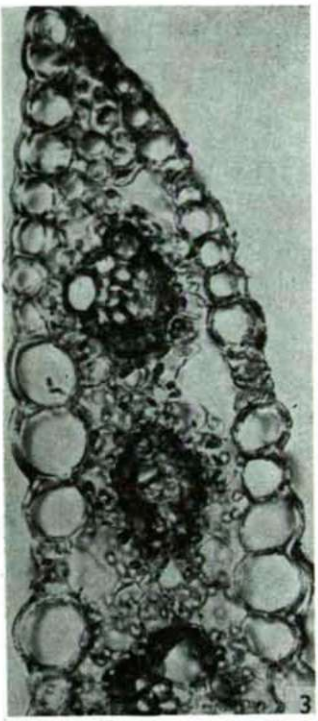


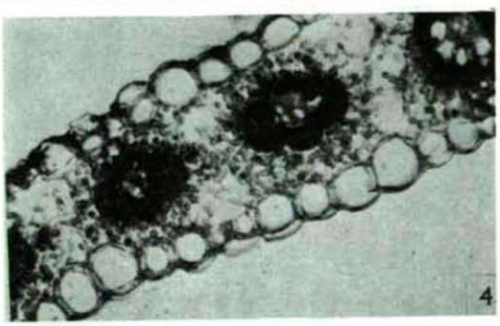
Picture 1: P. 1. 165, Picture 2: P. 1. 523, strongly increased bundle sheath chloroplasts of identical magnification (x1000). Pictures of the cross-section (Picture 3: P. 1. 165) and of the longitudinal section (Picture 4: P. 1. 523) of bundle sheath chloroplasts of centrifugal arrangement, full of starch, of identical magnification (x1580).

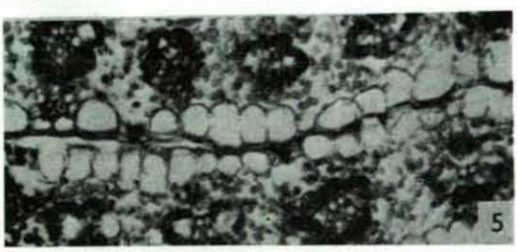
Plate V

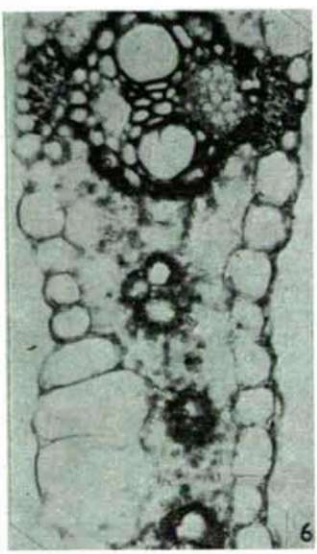












Picture 1, Picture 2: P. 1. 523. Leaf cross-section of 16–8 hrs LDC, of magnifications x163 and x645. Picture 3: P. 1. 523. Leaf cross-section of 30–15 min. LLC. x416. Picture 4: P. 1. (magnification: x253) and Picture 5: P. 1. 165 (magnification: x204). Cross-section of leaves of identical light-treatment (30–15 min. LDC). Picture 6: P. 1. 523. 15–7.5 min. LDC. Cross-section of a leaf. Magnification: x253.

matter quantity is the smallest. According to the literature — NOBEL, 1967; MURAKAMI and PACKER, 1970b, MUSTÁRDY, 1981 — the authors suppose that the considerable swelling of the Mchp of line 165 (in 30–15 min. LDC) originates from the thickening of granum membranes. Owing to the often repeated dark periods:

— On the two sides of thylakoid, the necessary proton gradient is not formed. The ATP formation decreases (HIND and JAGENDORF, 1963; PICK et al., 1973).

— The Mg^{2+}/H^{+} exchange between the loculus and partition is defective. According to BARBER (1976), BARBER and CROW (1972), the Mg^{2+} bond between the light. harvesting complex carboxyl groups, which is important from the view point of membrane-adhesion, does not take place, resp. it is but partial.

— According to MURAKAMI et PACKER (1970b), the grana are swelling because the carboxyl groups in the membranes (R-COO-) are hydrated. The question rises, why corn line 523 responds just opposite to the 30–15 min. LDC.

According to our supposition, in the Mchp of line 523, at the applied 32 Wm^{-2} light intensity, the necessary proton gradient is earlier formed. Our hypothesis (MARÓTI et. al., 1981) is supported by the following facts:

a) The — loculus pH-depending — xantophyll cycle of chloroplasts 523 is faster than that of line 165.

b) On the basis of the quantity piaments (chlorophyll b, lutein, neoxanthin) of the light-harvesting Chl a/b-protein complex (LHC), the volume of a chloroplast granum-loculus is, in case of corn line 165, much larger than that of line 523. Therefore, the determined pH difference (5–9) between the closed inner and outer membrane-spaces of Mchp 165 is formed comparatively slower.

c) The shrinkage of the Mchp, induced by the 30–15 min. LLC of continuous illumination, as well as the high value of the dry matter production seem to support our exception that the damaging effect of short rhythms originates from the proton emission of the frequently repeated dark period (the intrathylakoid space).

But the final decision of these questions, requires further many-sided investigations.

The uniform decrease in the size of Mchp in the 15–7.5 min. LDC originates from the — supposedly more increased — membrane destruction. It is shown by our pigment investigations (MARÓTI et al., 1981) that primarily the light-harvesting Chl a/b-protein complex is damaged.

The size of Bchp shows a positive connection with the quantity of dry matter. It is considerably determined by the number and size of the grains of starch in it.

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DORMANCY IN FRUITS OF THE TILIA PLATYPHYLLOS SCOP. VI. POSSIBLE ROLE OF THE EXOGENOUS GA_3 IN THE BREAKING OF DORMANCY

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Summary

Intensity of the respiration of the chilled-stratified seeds increases more intensively than that of the warm-stratified ones. Intensity of the respiration was further increased by treatment with exogenous GA_3 at both temperatures. Change in the lipase activity was similar. Embryos of seeds treated with exogenous GA_3 were larger than the untreated ones at both temperatures investigated. Slight differences were, however, observed in the germination rate of the excised embryos and in the intensity of the growth of the seedlings.

Faster degradation of the endosperm cells around the radicle was promoted by the chilled-stratification. This process cannot be forced by the exogenous GA_3 alone.

Introduction

In the seeds of the *Tilia platyphyllos* the embryo increases during the chilled-stratification (NAGY et al., 1981) and all changes promoting the germination take place.

The germination is an energy-intensive process since it includes the cell-division and elongation. Therefore, at the swelling of the seeds mobilization of the stored nutriments, which are utilized as respiratory basic materials and for the synthesis of new substances, starts immediately. In the mobilization of the nutriment taking place during the swelling of the seeds have an important role the hormones, firts of all the gibberellins which are important in the start of the synthesis and the enhancement of the activity of the hydrolytic enzymes of decisive importance (LEWAK et al., 1975; ZARSKA-MACIEJEWSKA and LEWAK, 1976). This may explain that in the case of numerous species the chilled-stratification requirement, necessary for the germination, can be substituted for exogenous GA_3 treatment (AMEN, 1968; JUNTTILA, 1970; ROSS and BRADBEER, 1971; KOPCEWICZ and PORAZINSKI, 1973; BASKIN and BASKIN 1970, 1974).

Since in the case of the *Tilia platyphyllos* seeds the chilled-stratification cannot be substituted for exogenous GA_3 treatment (NAGY and SZALAI, 1973), the aim of the present studies was to get further data concerning the influence of the exogenous GA_3 treatment on the dormancy of the *Tilia platyphyllos* seeds. That's why the effect of the GA_3 treatment was investigated concerning the metabolism of the seeds, the growing intensity of embryos excised from the treated seeds and the mechanical resistance of the tissues surrounding the embryo.

Materials and Methods

Fruits used for these studies were obtained from the Forestry of Csongrád County from trees forming close stand, thus population material was investigated.

The pericarps were mechanically removed and the seeds treated with sulfuric acid for 8 min then thoroughly washed, dried and put into filter-papers moistened water and 3×10^{-4} M GA_3 solution (Phylaxia, Budapest) and stored in refrigerator (at +5 °C), while an other series in thermostat of 25 °C.

Measurement of the intensity of the respiration

Intensity of the respiration of variously treated seeds was measured with Warburg's apparatus (KEIL and SORMOWA, 1968) in 3 weeks periods. Test pots were put into water-bath of 25 °C. The manometers were read at 15 min intervals for 60 min. Amount of oxygen consumed by the seeds was calculated from the following formula:

$$\mu l\ O_2 = k \cdot K_{\theta_2}$$

where k is the pressure measured with the manometer and K_{θ_2} is the factor of vessel belonging to the corresponding manometer. Three parallel series were used for the measurements. .

Measurement of the lipase activity

The enzyme preparations were made according to COLOWICK and KAPLAN (1955). Lipolytic activity was measured by using the method of ORY et al. (1962) after the determination of the pH optimum.

Measurement of the growth intensity of the excised embryos

In the different periods of the stratification the embryos were excised and stored in semisterile conditions in Petri dishes on filter-papers moistened with White's culture medium (WHITE, 1943) and illuminated with 10 000 lx for 16 h per day at 25 °C daily and 20 °C night temperature. Size of the seedlings was measured on the 3rd day after the excision.

Histological examination of the seeds

For the histological examination of the seeds, longitudinal sections were prepared by means of the usual paraffine embedding technique from seeds chilled-stratified for 3 months and from seeds warm-stratified for 6 months and treated with GA_3. The sections were stained with Ehrlich's acidic haematoxilin, fixed in glycerine-gelatine and examined with light-microscope.

Results and Discussion

Change in the intensity of the respiration of seeds under chilled- and warm-stratification

The intensity of the respiration measured with Warburg's apparatus with manometer is a good index of the activity of the metabolism.

Measure of the intensity of the respiration of the variously treated seeds at

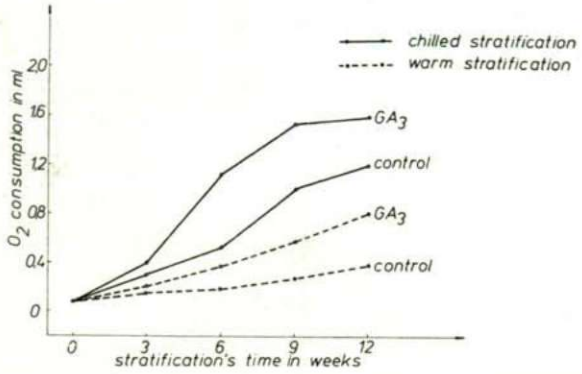

Fig. 1. Change of the respiration intensity of the *Tilia platyphyllos* seeds as a result of GA_3 treatment combined with chilled- and warm-stratification.

different times is demonstrated by Figure 1 showing the amount of oxygen (ml) consumed by 100 seeds during 1 h. As it can be seen from the Figure, intensity of the respiration of the warm-stratified seeds is much lower than that of the chilled-stratified ones.

The experiences are similar for the seeds of the pear (ALSCHER-HERMAN et al., 1981) and of the cherry too (POLLOCK and OLNEY, 1959). The intensity of the respiration is increased by the GA_3 treatment at both temperatures showing the metabolism increasing effect.

Change of the lipase activity of the seeds under chilled- and warm-stratification

In lipid-containing seeds the first step of the mobilization of the reserve materials is the degradation of the lipids with the help of lipases. Since the main reserve materials of the *Tilia* seeds are lipids (RADECKE, 1967), lipase activity was measured to characterize the metabolism activity.

Types of lipases present in the seed in dormancy and formed during the germination are question under dispute in the literature.

In the endosperm of the castor-oil bean a lipase with 4.3 pH optimum was found by ORY et al. (1962), while YAMADA (1957) described an other lipase system formed during the germination of the seed and possessing a pH optimum in the neutral range.

In the seeds, the presence of lipase system with different pH optima is contested by several authors. According to RAMAKRISHNAN and BANERJEE (1951) only one lipase system exists in the seeds pH optimum of which is in the acidic range, pH optimum observed in the neutral range is caused by the lipase activity of the microorganisms, invisible fungi, found on the seeds. This assumption was corroborated by the data of RIMON (1957) and ST ANGELO and ALTSCHUL (1964) as well.

However, lipase systems with different pH optima were found by SMOLENSKA and LEWAK (1974) in various seeds.

Determination of the pH optimum of the lipase activity of the *Tilia* seeds revealed two pH optima at 4.5 and 6.5, therefore, the activity was measured at both optima in all cases.

Change of the lipase activity of the *Tilia* seeds under stratification is shown by Figure. 2 (A and B). In the Figure, amount of the fatty acid (in oleic acid equivalent) released during 96 h by lipase extracted from 100 seeds is shown.

As it can be seen, during the 12 weeks investigation, the increase in the lipase activity of seeds stratified at room temperature was much lower than that of the chilled-stratified ones. The increase of the activity was observed at both pH as a result of the GA_3 treatment which is in accordance with the results of the measurements of the respiration intensity. The activity was higher at pH 6.5 (Fig. 2A) than at pH 4.5 (Fig. 2B).

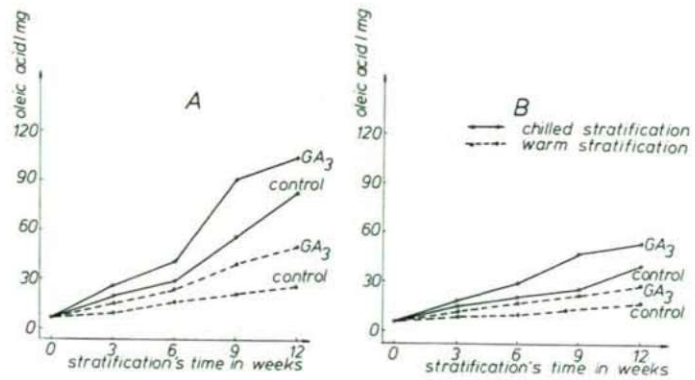

Fig. 2. Change of the lipase activity of the *Tilia platyphyllos* seeds as a result of GA_3 treatment at pH 6.5 (A) and pH 4.5 (B) combined with chilled- and warm-stratification.

Effect of the GA_3 treatment of the seeds on the growth of the embryos

Since in the case of the *Tilia platyphyllos* seeds the exogenous GA_3 treatment does not substitute the chilled-stratification, one can assume that amount of GA_3 necessary for the stimulation of the growth of the embryo cannot get into the seed. For this reason, embryos were excised from seeds treated with GA_3 for 2 months and compared with those excised from control seeds (Fig. 3). Figure shows the control embryos in the upper line while those excised from seeds treated with GA_3 in the lower line.

Although the embryos were larger than the control at both temperatures as a result of the exogenous GA_3 treatment, germination took place only in the case of the chilled-stratified seeds, and no germination was observed with the warm-stratified ones even on 6 months GA_3 treatment. The exogenous GA_3 treatment stimulated mainly the growth of the cotyledones. The same found for the Fraxinus excelsior seeds where, as a result of the GA_3 treatment, the cotyledones of the embryo increased to such an extent that they got crushed in the seed but no germination took place (SZALAI and NAGY, 1968).

Germination of the embryos excised from seeds treated with GA_3 under chilled- or warm-stratification was fast but the differences of the size of the seedlings did not justify the failure of the germination at room temperature. Figure 4 shows the size of the seedlings developed from the embryos obtained from variously treated seeds, 3 days after the excision. In the case of warm-stratification the resistance of the

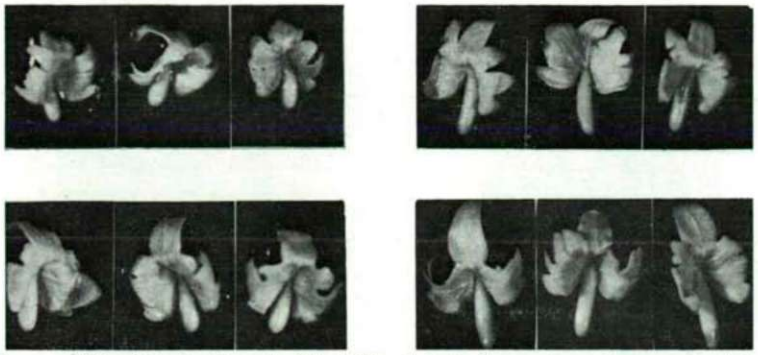

Fig. 3. Influence of the GA_3 treatment of the *Tilia platyphyllos* seeds on the growth of the embryos. Upper line is the control and the lower line shows the embryos excised from seeds treated with GA_3 for 2 months.

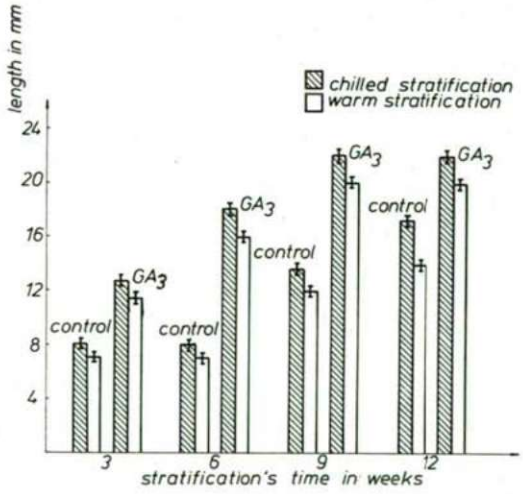

Fig. 4. Growing intensity of the embryos, excised from seeds treated with GA_3, at 25 °C and illuminated with 10 000 lx. Figure shows the size of the seedlings 3 days after the excision.

covering tissues seems to be the primary reason for the continuance of the dormancy and not the weaker growing ability of the embryo. This was corroborated by our histological observations as well.

Effect of the GA_3 treatment on the endosperm surrounding the embryo

At the end of the 3 months chilled-stratification the discharge and dissolution of the endosperm cells around the radicle were observed (Fig. 5). This change was not observed for warm-stratified and GA_3 treated seeds even after 6 months. This difference is essential concerning the cessation of the dormancy.

In the interuption of the dormancy the exogenous gibberellin would be effective if its role were double in the *Tilia platyphyllos* seeds: supply of the embryo with soluble nutriments and the stimulation of the synthesis and/or the activity of enzymes

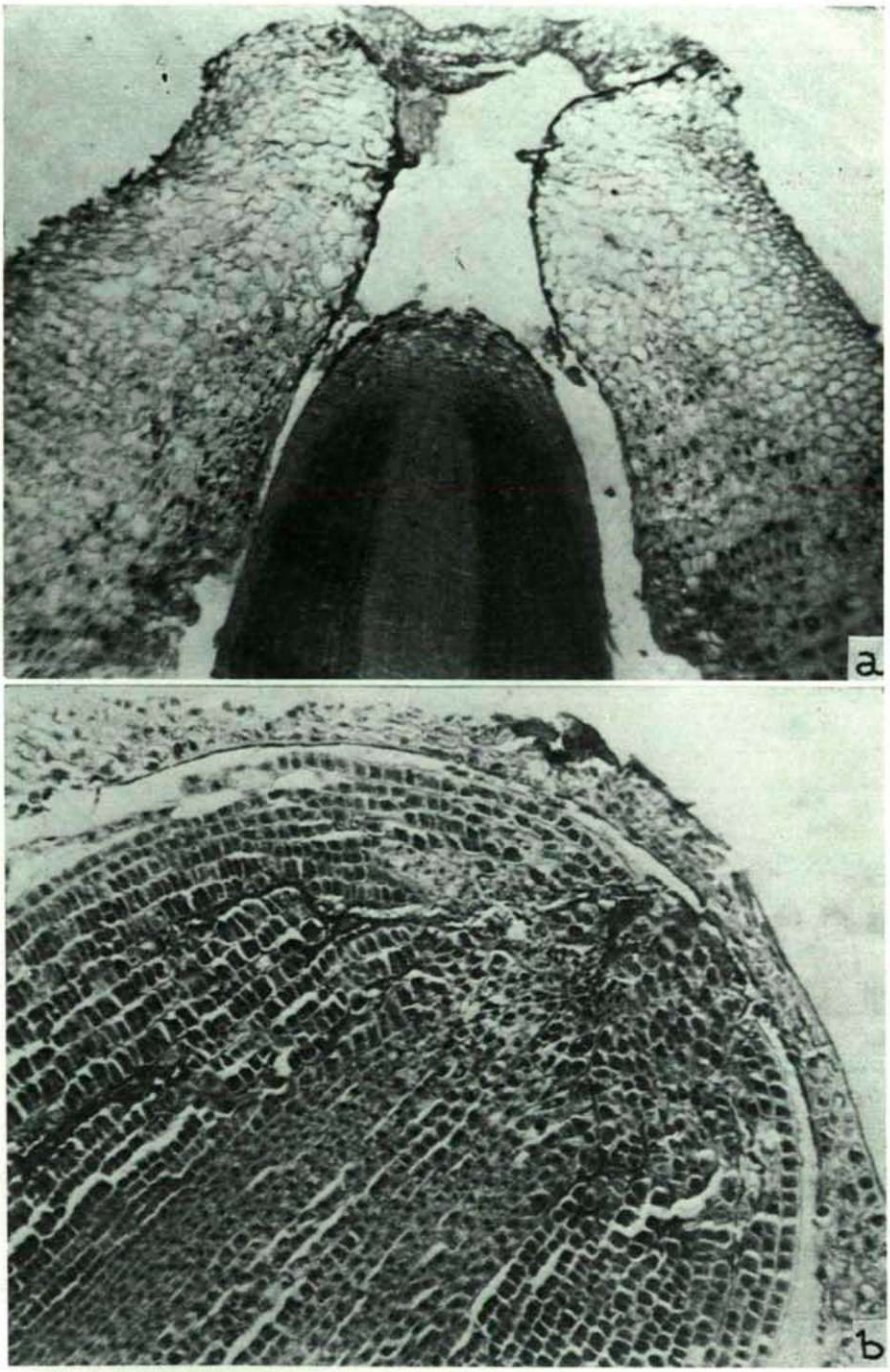

Fig. 5. Effect of GA_3 treatment on the endosperm around the radicle.
a) seed chilled-stratified for 3 months, x50.
b) seed treated with GA_3 and warm-stratified for 6 months, x123.

decreasing the mechanical resistance of tissues surrounding the embryo. As it is shown by our results, the enhancement on the gibberellin level is not enough for the cessation of the dormancy of the *Tilia* seeds since it can promote only the nutriment supply and the growth of the embryo. Probably the enhancement of the level of other hormones are also required for the weakening of the mechanical resistance of the endosperm.

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ON THE FAUNA OF THE SANDY SOIL GRASSLAND AT BUGAC

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Abstract

59 217 insects were collected by emergence traps in an ungrazed semi-natural grassland from 1976 to 1979 in the Kiskunság National Park ("KNP", Hungary). Qualitative and quantitative relations of the material collected are discussed on order level. Summing up the four year results p. c. distribution of the fauna is as follows: Hymenoptera 23.9%; Homoptera 16.6%; Diptera 10.5%; Acari 7.7% and Coleoptera 5.3%. In average 6795 individuals per sq. m. were collected during whole season with a maximum of 11 875 ind. per m^2. The distribution, weather hazards, dependent fluctuation as well as the importance of constant and changing traps were also examined. In unfavourable dry periods the majority of animals could leave the area. Owing to good environmental conditions individuals having immigrated from surrounding pastures also survived. 39% of the individuals examined developed in deeper wind furrows. Diplopoda, Thysanoptera, Lepidoptera and Araneida groups prefer wind furrows while Orthoptera, Homoptera, Hymenoptera and Acari were mostly collected on sand dunes. This preference depends on the season, as well. The following seasonal maxima of the important orders were established: Orthoptera: early summer; Cicadinea: early and late summer; Lepidoptera: May-June and August–September; Heteroptera: July.

Introduction

One of the most characteristical natural conservancy areas of Kiskunság National Park (KNP) is the semi-natural grassland in the neighbourhood of Kecskemét, Jakabszállás and Bugac. This grazed grassland covers about 2000 ha and a smaller plot was isolated for ecological investigations at the eastern edge of it.

The area consists of sand dunes and wind furrows that are sometimes 2 m deep (Fig. 1 and 2) having different microclimatical conditions and plant communities (KÖRMÖCZI, BODROGKÖZY and HORVÁTH, 1981). Sand dunes are covered by Festucetum vaginatae danubiale normale, *Potentillo-Festucetum pseudovinae danubiale euphorbietosum seguerianae* and its *Bromus tectorum* facies. In wind furrows (Fig. 2) *Lolio-Potentilletum anserinae* can be found with *Festuca pseudovina* facies (BODROGKÖZY and FARKAS, 1981).

We suppose that the most important factor for the knowledge of basic fauna is to investigate the animals having developed and active in the same area although there are a lot of faunistic elements that immigrate from a natural landscape with sand dunes belonging to KNP as well, from a forest at the edge of sampling area or perhaps from agricultural lands. This paper is a qualitative and quantitative elaboration of the material collected for four years and containing animals having developed in the investigation area. Further elaborations and evaluations are due in the future.

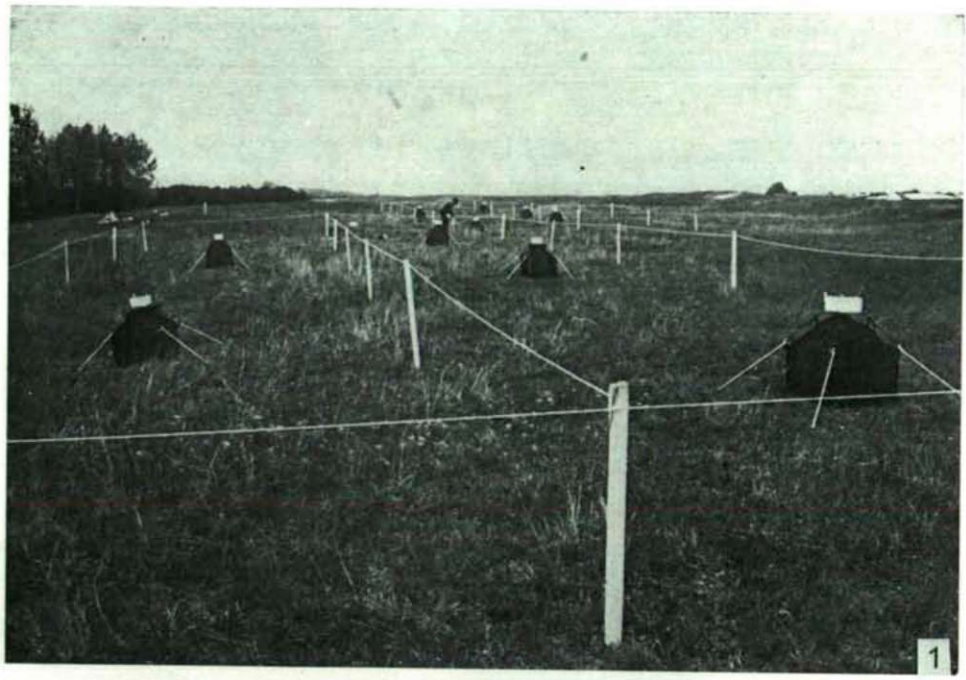

Fig. 1. Ten emergence traps in the natural conservancy area of Kiskunság National Park.

Fig. 2. Emergence traps on the light-coloured sand dunes and in the darker wind furrows.

There are several works on the fauna of typical Bugac area (FRIDVALSZKY, 1892; BIRÓ, 1896; MÓCZÁR, 1938; 1942; 1943; GOZMÁNY, 1954; SZELÉNYI, 1957; NAGY, 1958; KIS-ÚJHELYI, 1965; TÓTH, 1967; SZELÉNYI–NAGY–SÁRINGER, 1974; PAPP, 1975; VOJNITS, 1976). No other work has been made in Hungary with the methods used in this work with an exception of MÓCZÁR and BIRÓ (1980).

Methods

Emergence trap was introduced by SOUTHWOOD (1971) and observed in 1955 in Central High Alps: Obergurg area. It is the most useful to collect insects developing in the soil and in the plant layer. Janetschek and coworkers (1977) used these traps to observe the fauna of meadows at different heights. The basic area of this trap is 50 by 50 cm. The collection space is isolated by black tulle on an iron frame. Traps were dig some centimeters into the soil. In the fourth year bronze nets were also used and no modifications were observed in the collected material. Collecting vessels were in 60 cm height (Fig. 1, 2) and in the centre of the soil covered by emergence trap a Barber trap was placed. 50% ethylene glycol was used in both types of traps and were emptied in every month. Ten traps were used in every year from 28th April to 21st October in 1976, from 31st March to 3rd November in 1977, from 15th March to 16th November in 1978 and from 15th March to 5th November in 1979, so altogether 580 samples were collected. Five traps were used permanently in the same place and five were transplanted in every month within an area of 25 m². Traps were put in the deeper parts of the area with dense vegetation cover, too (Fig. 2). We used these two types of traps because the number of animals developing tn unit area could be estimated on the basis of the material of permanent traps and changing iraps collected the animals occuring in the trapped area. Differences between these two types

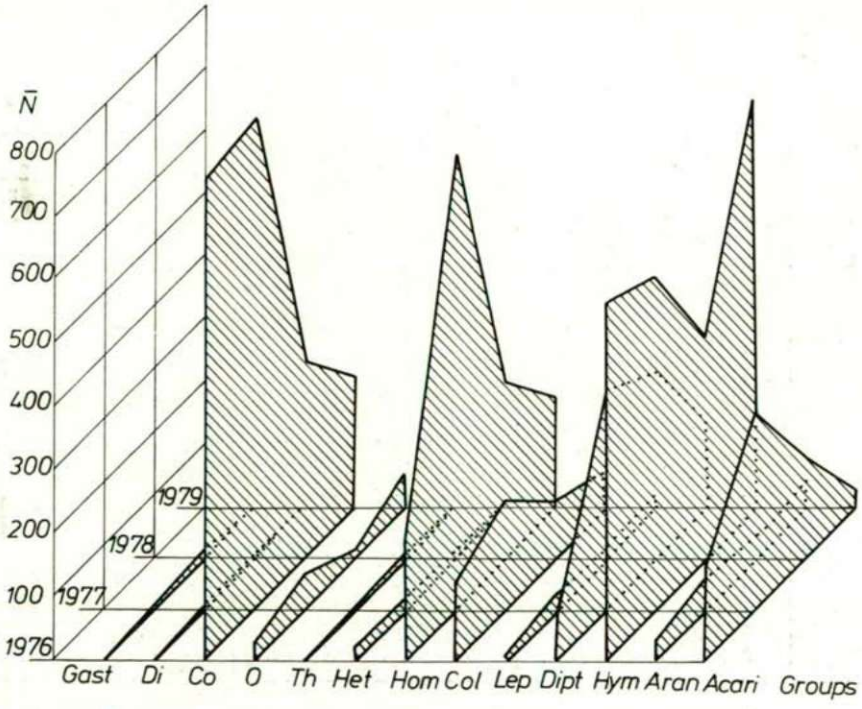

Fig. 3. Annual fluctuations in average number of important taxonomic groups.

were caused by the different life span of animals (depending on the role of predators, traps etc.) and the influence of surrounding areas (migration). Otherwise the effeciency of captures cannot be 100% because populations of certain species can avoide to get into the collecting solution (e.g. species with reduced locomotion activity).

P. KESZEI and F. ACSAI helped in handling traps, collected materials were preserved and handled by ANNA FAZEKAS, and Dr. GABRIELLA SZŐNYI-DOMOKI helped in evaluating the collection data. Authors are grateful for their valuable help.

Taxonomical distribution of material collected

The total number of specimens collected in the 290 sample during four years was 59 257 (Table I). Groups that have less than 100 specimens were not evalueted in the Table I. The percentage data of the taxonomic groups in the Table correspond to the total number of specimens collected during the whole four year period in the constant and changing traps. The p.c. group distribution of the total collected material can be seen in the last column. The most numerous groups are: Hymenoptera: 14 981 specimens, 25.3% (mainly ants); Collembola: 14 142 specimens, 23.88% and Homoptera: 9814 specimens, 16.58% (mainly Aphids). The maximum number of ants is due to immigration into the trapped areas. The high number of Collembola

Table 1. Annual distribution of mean number in collected material.

	1976				1977				1978			
	c		v		c		v		c		v	
	N	%	N	%	N	%	N	%	N	%	N	%
Di	1	0.98	—	—	15	14.71	13	12.75	26	25.49	35	34.31
Co	2898	20.49	1631	11.53	2706	19.13	2789	19.72	1251	8.85	1208	8.54
O	64	5.94	105	9.75	219	20.33	168	15.6	32	2.97	58	5.39
Th	3	2.0	11	7.33	37	24.67	15	10.0	28	18.67	38	25.33
Het	43	10.14	71	16.75	81	19.1	51	12.03	79	18.63	38	8.96
Hom	854	8.7	269	2.74	4359	44.4	681	6.94	1746	17.78	487	4.96
Col	206	6.59	529	16.92	670	21.43	537	17.17	340	10.87	364	11.64
Lep	29	5.46	40	7.53	91	17.14	86	16.19	56	10.55	62	11.68
Dipt	168	2.69	198	3.17	1254	20.09	1182	18.94	1175	18.82	1169	18.73
Hym	2225	14.85	1152	7.69	2003	13.37	1675	11.18	1150	7.68	1635	10.91
exc. Form	153	5.49	213	7.64	499	17.91	490	17.59	378	13.57	468	16.80
Aran	79	6.78	111	9.53	174	14.93	193	16.57	130	11.16	137	11.76
Acari	460	10.04	418	9.13	1403	30.63	757	16.52	712	15.54	578	12.62
Gast	2	2.04	—	—	1	1.02	4	4.08	19	19.4	61	62.24
Isop	—	—	3	5.88	21	41.18	1	1.96	21	41.18	1	1.96
Chil	—	—	—	—	—	—	1	16.67	4	66.66	—	—
Blat	8	11.59	4	5.8	5	7.25	13	18.84	23	33.33	11	15.94
Mant	—	—	1	33.33	—	—	—	—	—	—	1	33.33
Plec	—	—	2	100.0	—	—	—	—	—	—	—	—
Derm	1	100.0	—	—	—	—	—	—	—	—	—	—
Psoc	—	—	—	—	1	50.0	1	50.0	—	—	—	—
Neur	—	—	3	42.85	—	—	—	—	1	14.29	2	28.57
Trich	—	—	1	100.0	—	—	—	—	—	—	—	—
Inv	225	8.68	151	5.83	1581	61.0	335	12.92	12	0.46	17	0.66
Others	9	20.0	6	13.34	14	31.11	14	31.11	—	—	2	4.44
Total	7275	60.72	4706	39.27	14 635	63.21	8516	36.78	6805	51.21	5904	48.87

	1979				1976–79				1976–79	
	c		v		c		v		c+v	
	N	%	N	%	Σ N	%	Σ N	%	Σ N	↓%
Di	3	2.94	9	8.82	45	44.12	57	55.88	102	0.17
Co	296	2.1	1363	9.64	7151	50.57	6991	49.43	14142	23.88
O	249	23.12	182	16.9	564	52.37	513	47.63	1077	1.82
Th	8	5.33	10	6.67	76	50.67	74	49.33	150	0.25
Het	25	5.9	36	8.49	228	53.77	196	46.23	424	0.72
Hom	697	7.1	725	7.38	7656	77.98	2162	22.02	9818	16.58
Col	183	5.85	289	9.53	1399	44.74	1728	55.26	3127	5.28
Lep	55	10.36	112	21.09	231	43.5	300	56.5	531	0.90
Dipt	437	7.00	659	10.56	3034	48.61	3208	51.39	6242	10.54
Hym	2570	17.16	2571	17.16	7948	53.05	7033	46.95	14981	25.3
exc. Form	220	7.89	364	13.07	1250	44.88	1535	55.11	2785	4.7
Aran	148	12.7	193	16.57	531	45.58	634	54.42	1165	1.97
Acari	138	3 01	115	2.51	2713	59.22	1868	40.78	4581	7.73
Gast	1	1.02	10	10.2	23	23.47	75	76.53	98	0.17
Isop	—	—	4	7.84	42	82.35	9	17.65	51	0.09
Chil	—	—	1	16.67	4	66.66	2	33.34	6	0.01
Blat	—	—	5	7.25	36	52.17	33	47.83	69	0.11
Mant	1	33.33	—	—	1	33.33	2	66.67	3	0.005
Plec	—	—	—	—	—	—	2	100.0	2	0.003
Derm	—	—	—	—	1	100.0	—	—	1	0.001
Psoc	—	—	—	—	1	50.0	1	50.0	2	0.003
Neur	1	14.29	—	—	2	28.57	5	71.43	7	0.012
Trich	—	—	—	—	—	—	1	100.0	1	0.001
Inv	89	3.43	182	7.02	1907	73.57	685	26.43	2592	4.38
Others	—	—	—	—	23	51.11	22	48.89	45	0.075
Total	4901	43.08	6475	56.92	33 616	56.76	25 601	43.23	59 217	100

Table 2. Total annual number (N) and monthly average ($\bar{x}$) of important taxonomic groups.

Group	1976		1977		1978		1979	
	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$
Gastropoda	2	0.33	5	0.71	80	10.0	11	1.375
Diplopoda	1	0.17	28	4.0	61	7.625	12	1.5
Collembola	4529	754.83	5495	785.0	2459	307.375	1659	207.375
Orthoptera	169	28.17	387	55.28	90	11.25	431	53.875
Thysanoptera	14	2.33	52	7.42	66	8.25	18	2.25
Heteroptera	114	19.0	132	18.86	117	14.625	61	7.625
Homoptera	1123	187.17	5040	720.0	2233	279.125	1422	177.75
Coleoptera	735	122.5	1207	172.43	704	88.0	481	60.125
Lepidoptera	69	11.5	177	25.28	118	14.75	167	20.875
Diptera	366	61.0	2436	348.0	2344	293.0	1096	137.0
Hymenoptera	3377	562.83	3678	525.43	2785	348.125	5141	642.625
(exc Form.)	366	61.0	989	141.28	846	105.75	584	73.0
Araneidea	190	31.67	367	52.43	267	33.375	341	42.625
Acari	878	146.33	2160	308.57	1290	161.25	253	31.625

is reasonable, their number was more than ten times higher in sodic areas (MÓCZÁR and BIRÓ, 1980). 10.54 p.c. of the basic fauna is Diptera (6942 individuals) 7.73% (4581 specimens) are Acari and 5.28% (3127 specimens) are Coleoptera. The number of Acari is uncertain for technical reasons. With the exception of Araneidea (1165 specimens, 1.97%) and Orthoptera (1077, 1.82%) the other groups represent less than 1%.

Since the investigation periods were different for a more exact comparison the annual average of main groups is demonstrated, as well in Table II and Fig. 3.

Relations between climate and collected material

The following 8 groups had the highest average number of individuals in 1977: Collembola, Orthoptera, Homoptera, Coleoptera, Lepidoptera, Diptera, Araneidea and Acari. The mean temperature of the season was the highest in that year during the investigation period, 14.71 °C. The precipitation was 258 mm and it was very low comparing with the average (343 mm). There were semiarid conditions in July (Fig. 4) and there was a shortage of precipitation also in spring and autumn. This climate is advantageous for the development of above mentioned groups.

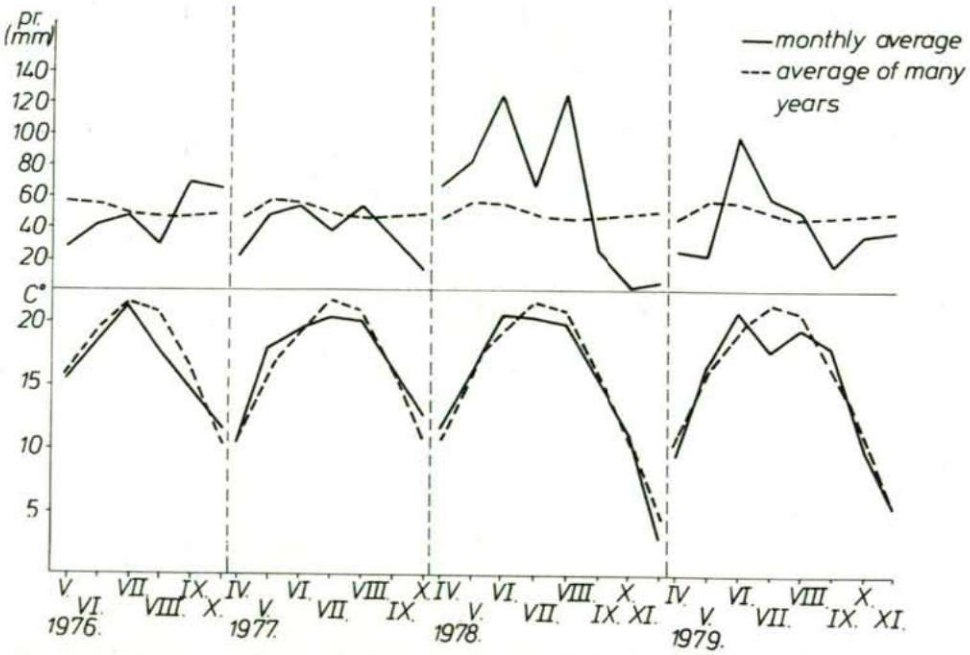

Fig. 4. Monthly average temperature and precipitation in the investigation period.

Five groups had the highest average in 1978: Gastropoda, Thysanoptera, Isopoda and Blattoidea (Table I). In that year the mean temperature was 14.08 °C, so fell between 1977 and 1979 average but the precipitation was unusually high in spring and summer, it reached 491 mm in the growing season that is 100 mm higher than the many year average. Since the highest precipitation was in July and August

it was especially advantageous for the vegetation. This is the reason of the high amount of hygrophilé animals.

Only Heteroptera had a maximum in 1976. Precipitation was 276 mm, 22 mm less than the average of many years and especially May and August were poor in precipitation. Average temperature was relatively low.

Hymenoptera was the only order having maximum in 1979. Average temperature of growing season was a little lower than in other years, 13.85 °C. Precipitation was 57 mm less than the average but it concentrated to the summer period. The water shortage in spring caused a two week delay in the development of the vegetation.

Not considering ants because of their special way of life, the number of collected individuals belonging to other Hymenoptera groups (mainly Terebrantes) was 2785 and it means maximum in the relation of the four years.

Upper and lower traps

On the basis of four year data Diplopoda, Collembola, Orthoptera and Acari were in greater proportion in Barber traps while the percentage ratio of Thysanoptera, Homoptera, Coleoptera, Lepidoptera and Diptera was higher in the upper traps. The quantity of Araneidea was almost the same in both types of traps (50.21 and 49.79% respectively) (Table 3.).

The details are not so unambigous. In the constant traps 51.22% was collected by the lower and only 48.78% by the upper traps. In the changing traps 48.58% was collected by the lower and 51.42% in the upper traps. The majority of Hymenoptera were in the lower traps because of the high proportion of ants. Majority of Heteroptera species was expected in the upper traps but their 57.08 percentage was represented in the lower traps and only 42.92% was collected in the upper traps so they were active mostly on the surface of soil and not on the vegetation.

There are some contradictory cases in the annual data, e.g. in 1977 only 36% of Diplopoda was collected in the lower and 64% in the upper traps. In 1976 and 1978 only 37–42% of the Homoptera was in the upper, 63–58% in the lower trap. It is obvious both in the global 4-year result and in the results of the upper and the lower traps respectively. The smaller amount appeared in the constantlower (41%), and in the changingupper traps (48%). The bigger amount of the Homoptera appeared in the constantupper (58%), and in the changing-lower traps (52%). It is obvious that the animals got into the traps set opposite to their living places when trying to escape. The contradictory phenomena mentioned above are the consequences of the fact that the number of species of the listed taxonomic categories are too big, the populations belong to different types of way of living. Only the accurate examination of these and the clarification to the species will lead to satisfactory results.

The constant and the changing traps

If we examine the last line of Table I where we summed the hived animals regardless to their proper taxonomic place, it will be clear that both in the global results of the 4 years (56.76%) and in the years 1976, 1977, 1978 (60.72%, 63.21%, 51.21%) the bigger amount was collected in the constant trap, while in 1979 the situation was just the opposite: only 43.08% of the animals got to the constant trap, the others (56.92%) got to the changing one.

Table 3. P. c. distribution between upper and lower traps.

Group		1976	1977	1978	1979	1976–79 Σ
Diplopoda	u	100	64.29	6.56	16.67	24.51
	l	—	35.71	93.44	83.33	75.49
Collembola	u	38.24	50.19	21.31	9.64	36.59
	l	61.76	49.81	78.69	90.36	63.41
Blattodea	u	83.33	83.33	82.35	60	81.16
	l	16.67	16.67	17.65	40	18.84
Orthoptera	u	42.6	30.75	22.22	19.03	27.2
	l	57.4	69.25	77.78	80.97	72.8
Thysanoptera	u	35.71	82.69	75.76	77.78	74.67
	l	64.29	17.31	24.24	22.22	25.33
Heteroptera	u	50	46.97	24.79	55.74	42.92
	l	50	53.03	75.21	44.26	57.08
Homoptera	u	36.51	72.88	42.36	37.41	56.64
	l	63.49	27.12	57.64	62.59	43.36
Coleoptera	u	48.03	63.79	74.29	54.68	61.05
	l	51.97	36.21	25.71	45.32	38.95
Lepidoptera	u	81.16	90.4	67.8	74.85	79.28
	l	18.84	9.6	32.2	25.15	20.72
Diptera	u	61.75	83.13	78.5	81.48	79.85
	l	38.25	16.87	21.5	18.52	20.15
Hymenoptera	u	23.22	36.27	33.79	40.75	34.4
	l	76.78	63.73	66.21	59.25	65.6
Araneidea	u	22.63	50.41	56.55	60.41	50.21
	l	77.37	49.59	43.45	39.59	49.79
Acari	u	27.33	33.19	23.41	27.67	29.01
	l	72.67	66.81	76.59	72.33	70.99

As regards the global number of specimens there were more entities of 7 taxonomic categories in the constant traps, than in the changing ones. These are: the Coleoptera, Orthoptera, Thysanoptera, Heteroptera, Homoptera, Hymenoptera, and Acari. In the changing traps the following 5 categories were represented in bigger number: Diplopoda, Coleoptera, Lepidoptera, Diptera, and Araneida. Besides the differences of the percentages of the same groups between the constant and the changing traps are not too significant, for example: Collembola, Thysanoptera, Diptera (C:V) 2%; Orthoptera 4%; Hymenoptera 7%; Heteroptera and Araneida 8%; Coleoptera 10%; Diplopoda 12%; Lepidoptera 13%; Acari 18%. It seems that according to the 4-year global data the separation has no significance. Only the Homoptera's presence is significant: there were 56% more in the constant traps than in the changing ones (77.98:22.2%). The possibly reason of this must be the quick increase in the number of the aphids. In the constant trap as a sealed space — during several months — their number can quickly grow in spite of the fact that those which had got to the destroying liquid were removed at the monthly emptyings,

but those which remained alive under the net could continue to multiplicate. However the animals always spread from the changing traps after a month.

If we look at the annual data, the differences are greater, there are even contradictory results. Where the 4-year global number was bigger in the constant trap, generally the changing trap caught more animals in two years. This fact may explain the relatively small differences of the 4-year results mentioned above by it. For example the Heteroptera:

1977=const. 61.36%: chang. 38.63% 1976=chang. 62.28%: const. 37.71%
1978=const. 67.52%: chang. 32.47% 1979=chang. 59.01%: const. 40.98%.

Relations of abundancy

The data reduced to specimen/m² are in Table IV. If we add up the number of entities caught by the constant traps, we get how many animals develop minimally from the group in question on a given area (1 m²). So the annual summary is important here. In the case of the changing trap, when it is put to a new place, the results can be decisively different because of the animals having already been there, and because of the eggs laid meanwhile. Besides, we must not ignore the free im- and outmigration before the translocation and the intense predation. If for example the migration is rather insignificant, the predation will be the main reason for the differences. If the immigration is intense or more generations are developing because of further egg laying, the number of individuals will be greater in the changing than in the constant trap. If the outmigration is more intense, the number of individuals will be greater in the constant trap than in the changing one. The data of the changing traps can be closer to real conditions, i.e. the reflect the amount of the populations that can be found and can survive on the area.

According to the constant separators a 4-year average of entities developing per m² are 6795 individuals. This amount varies year by year, the maximum, 11 875 was in the dry year of 1977 (Table IV). The May peak of the Collembola (918 p/m²) and the high number of entities of the Homoptera in June–July (930 and 1266 p/m²) contributes to this large number. The amount of the Hymenoptera (1602), the Acari (1122) and the Diptera is also significant. The 1976 datum (5842 p/m²) approaches the average, most while the number of animals per area has decreased since 1978 (5499 and 3963) specimens. This decrease is caused by the strong decrease of the Collembola (from 1001 to 237), the Homoptera (from 1397 to 558), the Diptera (from 940 to 349) and the Acari (from 570 to 110). The amount of the Hymenoptera (from 920 to 2056) and the Orthoptera (from 26 to 199) however increased. The majority of the animals grow till August.

From the connection between the constant and the changing traps we can draw a conclusion how the conditions of the areas in each year promoted the survival of the animals, when the mortality and migration were higher and how each group was influenced by them. The greatest difference between the traps was in 1977: under the changing traps we could find only half of the number found under the constant ones. In spite of the fact that the number of growing animals was the highest here, most of them possibly left the ares because of the draughty weather.

It is obvious in the case of the Homoptera. In 1978 however the difference was very small, the proportion even shifted in favour of the changing traps in special

Table 4. Monthly density data of important groups. 1976

Group	N/m²	V	VI	VII	VIII	IX	X	V–X	Monthly average
Di	c	—	—	0.8	—	—	—	0.8	0.13
	v	—	—	—	—	—	—	—	—
Co	c	415.6	619.2	445.6	212.0	38.4	736.0	2466.8	411.1
	v	415.6	472.8	52.8	130.4	60.8	24.0	1156.4	192.73
O	c	32.8	16.8	2.4	7.2	5.6	0.8	65.6	10.93
	v	32.8	16.0	16.8	3.2	0.8	—	69.6	11.6
Th	c	1.2	—	—	1.6	—	—	2.8	0.46
	v	1.2	0.8	0.8	4.0	—	1.6	8.4	1.40
Het	c	6.4	8.0	11.2	4.8	0.8	2.4	33.6	5.6
	v	6.4	13.6	11.2	9.6	8.0	8.8	57.6	9.6
Hom	c	41.2	96.8	60.8	377.6	32.0	80.0	688.0	114.6
	v	41.2	51.2	63.2	25.6	10.4	18.4	210.0	35.00
Col	c	146.4	44.8	20.0	15.2	6.4	12.8	245.6	40.93
	v	146.4	85.6	55.2	24.8	16.8	13.6	342.4	57.06
Lep	c	6.8	4.8	2.4	5.6	2.4	1.6	23.56	3.92
	v	6.8	8.0	2.4	12.0	1.6	0.8	20.2	3.36
Dipt	c	40.8	20.0	8.8	32.8	16.8	19.2	138.4	23.06
	v	40.8	44.0	31.2	19.2	2.4	16.8	154.4	25.73
Form	c	423.2	112.0	665.6	164.8	22.4	8.0	1396.0	232.7
	v	423.2	188.0	320.0	38.4	16.0	5.6	991.2	165.2
Hym	c	31.2	12.8	20.8	42.4	13.6	17.6	138.4	23.1
Others	v	31.2	30.4	40.0	39.2	16.0	19.2	176.0	29.3
Aran	c	18.0	13.6	10.4	9.6	7.2	3.2	61.98	10.33
	v	18.0	19.2	32.8	16.0	1.6	2.4	90.0	15.0
Acari	c	64.4	28.8	49.6	101.6	16.8	129.6	390.8	65.13
	v	64.4	27.2	18.4	139.2	1.6	60.8	311.6	51.93
Others	c	38.8	15.2	56.8	40.8	16.0	21.6	190.0	31.66
	v	38.8	28.0	43.2	22.4	4.0	6.4	142.8	23.80
Total	c	1266.8	992.8	1354.4	1016.0	178.4	1032.8	5842.4	
	v	1266.8	984.8	688.0	484.0	140.0	178.4	3730.6	

Table 4. 1977

Group	N/m²	IV	V	VI	VII	VIII	IX	X	IV–X	Monthly average
Di	c	0.4	—	1.6	—	0.8	0.8	0.8	11.93	1.70
	v	0.4	0.8	0.8	—	0.8	1.6	6.4	10.40	1.48
Co	c	802.8	917.6	396.0	221.6	84.0	69.6	8.0	2499.6	357.08
	v	802.8	741.6	148.0	80.8	80.8	42.4	—	1896.4	270.91
O	c	5.2	35.2	72.8	28.0	23.2	8.8	1.6	174.8	24.97
	v	5.2	72.0	16.0	23.2	4.0	10.4	4.0	134.8	19.25
Th	c	1.2	0.8	7.2	6.4	6.4	5.6	1.6	29.1	4.15
	v	1.2	2.4	1.6	1.6	2.4	3.2	—	12.4	1.77
Het	c	0.8	5.6	20.8	12.8	12.8	6.4	5.6	64.7	9.25
	v	0.8	4.8	9.6	4.8	12.8	4.8	3.2	40.7	5.82
Hom	c	77.2	81.6	930.4	1266.4	227.2	550.4	357.6	3490.8	498.68
	v	77.2	241.6	72.0	17.6	60.8	52.0	20.0	541.2	77,31
Col	c	51.6	98.4	200.0	64.0	43.2	32.2	47.2	536.4	76.62
	v	51.6	222.4	52.8	26.4	24.0	24.8	27.2	429.2	61.31
Lep	c	—	20.8	24.8	2.4	12.8	10.4	1.6	72.8	10.4
	v	—	26.4	14.4	—	2.4	25.6	—	68.7	9.82
Dipt	c	154.4	171.2	322.4	167.2	124.8	69.6	56.0	1065.6	152.2
	v	154.4	198.4	259.2	88.8	59.2	53.6	69.6	883.2	126.17
Form	c	109.2	88.8	316.8	232.8	152.0	108.0	123.2	1130.8	161.5
	v	109.2	274.4	388.0	120.8	45.6	64.0	18.4	1020.4	145.8
Hym	c	25.6	45.6	72.8	71.2	66.4	85.6	40.8	408.0	58.3
Others	v	25.6	72.0	105.6	45.6	40.8	74.4	19.2	383.2	54.7
Aran	c	14.8	24.0	57.6	12.0	10.4	12.0	8.8	139.6	19.94
	v	14.8	18.4	63.2	24.8	9.6	16.0	7.2	154.0	22.00
Acari	c	271.2	301.6	173.6	151.2	55.2	60.0	24.8	1037.6	148.22
	v	271.2	212.0	76.8	35.2	25.6	64.0	5.6	690.4	98.62
Others	c	113.2	150.4	342.4	504.0	26.4	64.8	12.0	1213.0	173.28
	v	113.2	97.6	95.2	55.2	8.0	6.4	6.4	382.0	54.57
Total	c	1627.6	1941.6	2939.2	2740.0	845.6	1084.0	696.8	11 874.8	
	v	1627.6	2184.8	1303.2	524.8	376.8	443.2	187.2	6647.6	

Table 4.

1978

Group	N/m²	IV	V	VI	VII	VIII	IX	X	XI	IV–XI	Monthly average
Di	c	3.2	0.8	1.6	0.8	3.2	1.6	4.0	4.0	19.2	2.40
	v	3.2	—	—	—	9.6	2.4	7.2	7.2	24.0	3.0
Co	c	58.8	70.4	62.4	145.6	381.6	136.8	122.4	12.0	990.0	123.75
	v	58.8	89.6	28.8	176.8	327.2	110.4	168.8	16.8	977.2	122.15
O	c	—	1.6	4.0	14.4	1.6	2.4	1.6	—	25.6	3.20
	v	—	4.8	25.6	5.6	9.6	0.8	—	—	46.4	5.80
Th	c	2.4	8.8	2.4	4.8	4.0	—	—	—	22.4	2.80
	v	2.4	5.6	7.2	12.0	0.8	—	2.4	—	30.4	3.80
Het	c	1.6	2.4	0.8	12.8	29.6	12.0	2.4	—	61.6	7.7
	v	1.6	3.2	5.6	4.0	10.4	2.4	2.4	2.4	32.0	4.0
Hom	c	24.0	23.2	480.8	228.8	169.6	253.6	175.2	50.4	1405.6	175.7
	v	24.0	41.6	76.80	90.4	56.0	23.2	60.8	8.0	380.8	47.6
Col	c	29.2	39.2	48.0	42.4	40.8	33.6	33.6	7.2	274.0	34.25
	v	29.2	28.8	43.3	32.0	80.0	25.6	31.2	19.2	289.2	36.15
Lep	c	0.4	4.8	8.8	5.6	20.0	4.8	—	—	44.4	5.55
	v	0.4	9.6	8.8	4.8	17.6	4.8	0.8	3.2	50.0	6.25
Dipt	c	24.8	67.2	108.0	200.0	146.4	80.0	258.4	68.0	952.8	119.1
	v	24.8	22.4	141.6	130.4	227.2	63.2	284.8	28.0	922.4	115.3
Form	c	60.0	28.0	48.0	144.0	224.8	108.8	31.2	2.4	647.2	80.9
	v	60.0	127.2	176.0	173.6	263.6	68.0	31.2	4.8	904.0	113.0
Hym	c	5.2	6.4	31.2	36.8	81.6	63.2	48.8	26.4	299.6	37.45
Others	v	5.2	8.0	39.2	71.2	68.0	64.8	117.6	3.2	377.2	47.15
Aran	c	7.8	8.0	7.2	16.8	25.6	15.2	15.2	9.6	105.4	13.17
	v	7.8	14.4	6.4	19.2	25.6	12.0	12.8	9.6	107.8	13.47
Acari	c	78.8	23.2	91.2	126.4	123.2	49.6	84.0	12.0	587.6	73.45
	v	78.8	55.2	55.2	80.8	44.0	15.2	108.8	7.2	444.4	55.55
Others	c	0.4	0.8	9.6	4.8	24.0	15.2	7.2	1.6	63.6	7.95
	v	0.4	3.2	36.8	14.4	15.2	4.0	2.4	—	76.4	9.55
Total	c	295.8	284.8	904.0	984.0	1276.0	776.8	784.0	193.6	5499.0	
	v										

Table 4.

Group	N/m²	1979 IV	V	VI	VII	VIII	IX	X	XI	IV–XI	1976-1979 Monthly average		Monthly average
Di	c	0.8	—	—	—	—	—	1.6	—	2.4	0.30	4.53	1.13
	v	0.8	—	—	—	0.8	—	4.0	1.6	7.2	0.90	5.38	1.34
Co	c	58.0	22.4	24.8	0.8	40.8	48.8	24.8	16.0	235.6	29.45	921.38	230.34
	v	58.0	36.0	60.0	28.8	96.8	543.2	112.8	156.0	1091.6	136.45	722.24	180.56
O	c	—	25.6	130.4	24.8	13.6	3.2	1.6	—	199.2	24.90	64.0	16.0
	v	—	16.8	90.4	12.0	24.0	2.4	—	—	145.6	18.20	54.85	13.71
Th	c	—	1.6	2.4	0.8	0.8	—	0.8	—	6.4	0.80	8.21	2.05
	v	—	4.0	2.4	—	0.8	0.8	—	—	8.0	1.00	7.97	1.99
Het	c	—	1.6	4.8	4.8	6.4	—	2.4	—	20.0	2.50	25.05	6.26
	v	—	7.2	12.0	—	—	0.8	—	8.8	28.8	3.60	23.02	5.75
Hom	c	14.4	48.8	244.0	16.8	14.4	23.2	67.2	128.0	556.8	69.6	858.58	214.64
	v	14.4	20.0	186.4	80.0	75.2	89.6	44.0	71.2	580.8	72.60	232.41	58.10
Col	c	24.4	8.8	56.0	18.4	10.4	10.4	3.2	16.8	156.4	19.55	171.35	42.83
	v	24.4	15.2	79.2	20.8	11.2	35.2	17.6	24.8	228.0	28.50	183.02	45.75
Lep	c	0.4	12.8	24.8	0.8	1.6	3.2	0.8	—	44.4	5.55	25.42	6.35
	v	0.4	13.6	28.8	4.0	17.6	22.4	2.4	—	89.2	11.17	30.60	7.65
Dipt	c	68.0	55.2	57.6	67.2	39.2	20.8	24.0	12.8	344.8	43.10	337.46	84.36
	v	68.0	55.2	52.8	123.2	91.2	72.0	53.6	16.0	532.0	66.50	333.7	83.42
Form	c	71.2	241.6	308.8	724.8	529.6	22.4	16.0	1.6	1916.0	239.5	714.6	178.65
	v	71.2	453.6	492.0	382.4	176.8	72.8	27.2	53.6	1729.6	216.2	640.2	160.05
Hym	c	1.6	7.2	32.8	4.8	13.6	53.6	55.2	7.2	176.0	22.0	140.85	35.21
Others	v	1.6	35.2	55.2	15.2	29.6	67.2	78.4	8.8	291.2	36.4	167.55	44.88
Aran	c	12.0	8.8	15.2	13.6	14.4	31.2	20.8	4.8	120.8	15.1	58.54	14.63
	v	12.0	28.0	24.0	8.0	21.6	25.6	18.4	14.4	152.0	19.0	69.47	17.36
Acari	c	16.4	6.4	52.8	4.8	2.4	4.8	11.2	11.2	110.0	13.75	300.55	75.13
	v	16.4	10.4	8.8	—	—	24.0	10.4	22.4	92.4	11.55	217.65	54.41
Others	c	5.2	28.0	38.4	0.8	—	0.8	—	0.8	74.0	9.25	222.14	55.53
	v	5.2	131.2	13.6	4.8	0.8	2.4	—	3.2	161.2	20.15	108.07	27.01
Total	c	272.4	468.8	992.8	883.2	694.4	222.4	229.6	199.2	3962.8			
	v	272.4	826.4	1105.6	679.2	546.4	958.4	368.8	380.8	5138.0			

cases, because of favourable rainy weather. So not only the survival of growing insects is for sure, but also the entities arriving from the surrounding pastures can find the conditions necessary to their existence.

Distribution according to space levels

The examined area can be distributed into two levels: the sand dune with higher, dryer microclimate and the lower, wet windfurrow. The fauna of the space levels, the number of growing entities on a unit area are possibly different because of different natural conditions, vegetation cover and phytocenosis. Although we didn't get to analysis according to species, it seems practical to mention the differences found in the case of each group (Table V). Since the traps have been set up on both levels only from 1977, the data of 1976 are hot shown in this Table.

Examining the global data of constant traps it can be stated that 39% of the examined Arthropoda were growing in the windfurrow, as regards the average of 3 years. It is different in each group of course. For example the Collembola, the Heteroptera the Coleoptera and the Diptera are approximately distributed equally between the two levels. The Diplopoda, the Thysanoptera, the Lepidoptera and the Araneidea prefer the wind-furrow, while the Orthoptera, the Homoptera, the Hymenoptera and the Acari orders prefer the sand dunes. The meteorological factors can change the exact numerical ratios, what can be illustrated by the data of the rainy 1978.

The data coming from the changing traps show different proportions. The proportion of the individuals of the Hymenoptera and the Acari moving in the windfurrows increased, it did not change in the case of the Diplopoda, Orthoptera, Homoptera, Coleoptera, Diptera and Araneida; and decreased in the cases of the Collembola, Thysanoptera, Heteroptera, and Lepidoptera orders. On an annual average Hymenoptera and Acarida growing in the sand dunes willingly make use of windfurrows, while the Collembola, Thysanoptera, Heteroptera and Lepidoptera growing in wind-furrows prefer sand dunes. It does not mean however that the other populations remain in their place of development during the whole year, because they can also react seasonally to the changes of conditions with migration, and significant deviation from the average can be observed in both directions.

The Araneida which generally prefer the wind-furrow, prefer the sand dune in spring and at the beginning of summer, while in the end of summer the proportion shifts in favour of the wind-furrow. The Acarida behave similarly. The spring peak of the Orthoptera populations is in the sand dune, in summer the majority prefer the wind-furrow, while in autumn they dominate in the sand duna again. The same type of dynamics can be recognised in the case of the Collembola. More Diptera appear in the windfurrow in spring, in summer the difference is balanced, while in the autumn of 1979 there were more of them in the sand dunes. The draught-resistance and the need of the Heteroptera forwarm are shown by the fact that double peak appear on both levels, but the mid-summer decrease is smaller in the sand dune than in the wind-furrow, i.e. the proportion is shifted in favour of the higher space level.

Table 5. Monthly density data in wind furrows (Wf) and sand dunes (Sh) in the years 1977–1979.

1977

		Di	Co	O	Th	Het	Hom	Col	Lep	Dipt	Hym	Aran	Acari	Others	Total
April	c Wf	2	406	—	4	2	38	46	—	138	274	24	336	262	1532
	Sh	—	509.3	9.3	—	—	97.3	54.6	—	60	81.3	8	369.3	158.6	1347.7
	v Wf	—	408	—	4	—	16	60	—	592	12	8	124	56	1280
	Sh	—	1320	6	—	1	97	50	—	123	86	17	202	19	1921
May	c Wf	—	596	4	—	6	60	124	12	222	178	54	342	98	1695
	Sh	—	1132	56	1.3	5.3	96	81.3	26.6	137.3	105.3	4	274.6	185.3	2105
	v Wf	4	528	16	4	4	288	412	32	280	856	24	76	292	2816
	Sh	—	795	86	2	5	299	175	25	178	219	17	246	49	2096
June	c Wf	8	108	40	—	—	36	136	88	488	144	28	96	40	1212
	Sh	—	468	81	9	26	1154	216	9	281	451	65	193	418	3371
	v Wf	—	124	8	—	20	20	72	16	300	168	44	132	32	936
	Sh	1.3	154	18	2	7	85	48	14	249	575	68	63	109	1393.3
July	c Wf	—	64	12	8	8	24	89	8	352	76	12	64	180	896.
	Sh	—	261	32	6	14	1577	58	1	121	361	12	173	585	3201
	v Wf	—	154	42	4	2	20	44	—	186	268	44	44	56	864
	Sh	—	32	10.6	—	6.6	16	14.6	—	24	98.6	12	29.3	54.6	298.3
Aug	c Wf	4	56	52	28	4	96	52	56	220	180	20	28	24	820
	Sh	—	91	16	1	15	260	41	2	101	228	8	62	27	852
	v Wf	2	172	6	4	16	50	18	4	82	90	20	26	20	510
	Sh	—	24	2.6	1.3	10.6	68	28	1.3	44	84	2.6	25.3	—	291.7
Sept	c Wf	4	84	20	12	—	60	44	16	48	24	12	8	8	340
	Sh	—	66	6	4	8	673	29	9	75	236	12	73	79	1270
	v Wf	4	86	4	4	2	16	16	4	92	116	20	136	8	508
	Sh	—	13.3	14.6	2.6	6.6	76	30.6	40	28	153.3	13.3	16	5.3	399.6
Oct	c Wf	16	20	—	—	2	—	28	—	28	32	8	40	8	182
	Sh	8	5	2	2	6	447	52	2	63	197	9	21	13	827
	v Wf	12	—	4	—	—	16	24	—	76	20	12	8	—	172
	Sh	6.6	—	4	—	4	21	28	—	68	42	6	5	7	191.6
April–Oct	c Wf	34	1334	128	52	22	314	518	180	1496	908	158	914	620	6678
	Sh	8	2532.3	202.3	23.3	74.3	4304.3	531.9	49.6	838.3	1659.6	118	1165.9	1465.9	12 973.7
	v Wf	22	1472	80	20	44	426	646	56	1608	1530	172	546	464	7086
	Sh	7.9	2338.3	141.8	7.9	40.8	662	374.2	80.3	714	1257.9	135.9	586.6	243.9	6591.5
Monthly average	c Wf	4.58	190.57	18.28	7.43	3.14	44.86	74	25.71	213.71	129.71	22.57	130.57	88.57	953.97
	Sh	1.14	361.75	28.9	3.33	10.61	614.9	75.98	7.08	119.76	237.08	16.86	166.55	209.41	1853.35
	v Wf	3.14	210.28	11.43	2.86	6.28	60.86	92.28	8	229.7	218.57	24.57	78	66.28	1012.25
	Sh	1.13	334.0	20.26	1.13	5.83	94.57	53.46	11.47	102	179.7	19.41	83.8	34.84	941.6

Table 5. 1978

		Di	Co	O	Th	Het	Hom	Col	Lep	Dipt	Hym	Aran	Acari	Others	Total
April	c Wf	8	126	—	4	4	10	38	2	14	44	10	20	—	280
	Sh	2.6	32	—	1.3	2.6	18.6	20	—	10.6	34.6	3.9	86.6	1.3	214.1
	v Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sh	1.6	48	—	2.4	—	32.8	31.2	—	37.6	92	9.6	96.0	—	351.2
May	c Wf	2	156	2	16	2	24	46	4	72	30	2	60	2	418
	Sh	—	13.3	1.3	3.9	2.6	22.6	34.6	5.3	64	37.3	12	12	—	208.9
	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sh	—	89.6	4.8	5.6	3.2	41.6	28.8	9.6	22.4	135.2	14.4	55.2	3.2	413.6
June	c Wf	4	64	—	2	2	68	68	8	98	62	2	132	14	524
	Sh	—	61.3	6.6	2.6	—	756	34.6	9.3	114.6	89.3	10.6	64	6.6	1155.5
	v Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sh	—	28.8	25.6	7.2	5.6	76.8	43.2	8.8	141.6	215.2	6.4	55.2	36.8	651.2
July	c Wf	2	194	20	4	22	116	46	6	184	344	24	74	4	1040
	Sh	—	113.3	10.6	5.3	6.6	304	40	5.3	210.6	72	12	161.3	5.3	946.3
	v Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sh	—	176.8	5.6	12.0	4.0	90.4	32.8	4.8	130.4	244.8	19.2	80.8	14.4	815.2
Aug	c Wf	8	706	2	8	62	186	52	20	200	172	32	98	24	1570
	Sh	—	165.3	1.3	1.3	8	146.6	33.3	20	110.6	396	21.3	141.3	23.9	1068.9
	v Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sh	9.6	327.2	9.6	0.8	10.4	56.0	80.8	17.6	227.2	331.2	25.6	44.0	14.0	1153.2

Sept	c	Wf	4	130	2	—	14	570	50	—	80	128	18	80	36	1112
		Sh	—	141.3	2.6	—	10.6	42.6	22.6	8	80	201.3	13.3	29.3	1.3	552.9
	v	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Sh	2.4	110.4	0.8	—	2.4	23.2	25.6	4.8	63.2	132.8	12.0	15.2	4.0	396.8
Oct	c	Wf	4	106	2	—	6	354	48	—	104	104	14	—	10	752
		Sh	4	133.3	1.3	—	—	56	24	—	361.3	64	16	140	5.3	805.2
	v	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Sh	7.2	168.8	—	2.4	2.4	60.8	31.2	0.8	284.8	148.8	12.8	108.8	2.4	831.2
Nov	c	Wf	2	6	—	—	—	82	4	—	12	24	14	19	2	156
		Sh	5.3	16	—	—	—	29.3	9.3	—	105.3	32	6.6	13.3	1.3	218.4
	v	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Sh	7.2	16.8	—	—	2.4	8.0	19.2	3.2	28.0	8.0	9.6	7.2	—	109.6
April–Nov	c	Wf	34	1488	28	34	112	1410	352	40	764	908	116	474	92	5852
		Sh	11.9	675.8	23.7	14.4	30.4	1375.7	218.4	47.9	1057	926.5	95.7	647.8	45	5170.2
	v	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Sh	28	966.4	46.4	30.4	30.4	389.6	291.2	49.6	935.2	1308.0	109.6	462.4	74.8	4722.0
Monthly average	c	Wf	4.25	186	3.5	4.25	14	176.25	44	5	95.5	113.5	14.5	59.25	11.5	731.5
		Sh	1.49	84.48	2.96	1.8	3.8	171.96	27.3	5.99	132.12	115.81	11.96	80.98	5.62	646.27
	v	Wf	—	—	—	—	—	—	—	—	—	—	—	—	—	—
		Sh	3.5	120.8	5.8	3.8	3.8	48.7	36.4	6.2	116.9	163.5	13.7	57.8	9.35	590.25

Table 5. 1979

		Di	Co	O	Th	Het	Hom	Col	Lep	Dipt	Hym	Aran	Acari	Others	Total
April	c Wf	2	70	—	—	—	12	10	—	52	4	16	26	2	194
	Sh	—	52	—	—	—	17.3	17.3	—	86.6	58.6	5.3	10.6	6.6	254.3
	v Wf	—	16	—	—	—	12	34	—	94	6	10	12	6	190
	Sh	1.3	84	—	—	—	14.6	34.6	1.3	42.6	177.3	17.3	18.6	5.3	396.9
May	c Wf	—	8	12	—	4	16	10	20	32	230	8	6	62	408
	Sh	—	32	34.6	2.6	—	70.6	8	8	70.6	261.3	9.3	6.6	5.3	508.9
	v Wf	—	44	16	—	—	—	4	2	20	52	16	8	32	194
	Sh	—	34	17	5	9	25	18	16	64	598	31	11	156	984
June	c Wf	—	12	76	2	2	182	58	22	56	314	20	38	40	822
	Sh	—	33.3	166.6	2.6	6.6	285.3	54.6	26.6	58.6	360	12	62.6	37.3	1106.1
	v Wf	—	—	84	4	—	32	32	4	12	132	16	24	—	340
	Sh	—	75	92	2	15	225	91	35	63	651	26	5	17	1297
July	c Wf	—	2	18	—	4	28	16	2	34	120	16	—	2	242
	Sh	—	—	29.3	1.3	5.3	9.3	20	—	89.3	1136	12	8	—	1310.5
	v Wf	—	16	4	—	—	12	—	—	96	304	12	—	4	448
	Sh	—	32	14	—	—	97	26	5	130	421	7	—	5	737
Aug	c Wf	—	52	4	—	2	18	6	4	32	62	10	6	—	196
	Sh	—	32	20	1.3	9.3	12	26.6	—	44	864	17.3	—	—	1026.5
	v Wf	4	60	12	4	—	—	8	72	92	88	8	—	—	348
	Sh	—	106	27	—	—	94	12	4	91	236	25	—	1	596
Sept	c Wf	—	32	2	—	—	20	4	4	4	44	50	—	—	160
	Sh	—	60	4	—	—	25.3	14.6	2.6	32	97.3	18.6	8	1.3	263.7
	v Wf	—	176	2	2	2	10	26	22	78	158	52	54	6	588
	Sh	—	788	2.6	—	—	142.6	41.3	22.6	68	128	8	4	—	1205.1

Oct	c	Wf	2	22	—	2	—	16	6	2	6	46	22	22	—	146
		Sh	1.3	26.6	2.6	—	4	101.3	1.3	—	36	88	20	4	—	285.1
	v	Wf	20	160	—	—	—	16	12	—	12	136	24	—	—	380
		Sh	—	101	—	—	—	51	19	3	64	98	17	13	1	367
Nov	c	Wf	—	14	—	—	—	30	6	—	8	2	6	2	—	68
		Sh	—	17.3	—	—	—	193.3	24	—	16	13.3	4	17.3	1.3	286.5
	v	Wf	8	84	—	—	—	4	48	—	12	212	12	36	8	424
		Sh	—	174	—	—	—	88	19	—	17	25	15	19	1	358
April–Nov	c	Wf	4	212	112	4	12	322	116	54	224	822	148	100	106	2236
	v	Wf	32	556	118	10	2	86	164	100	416	1088	150	134	56	2912
		Sh	1.3	1394	152.6	7	24	737.2	260.9	86.9	539.6	2334.3	146.3	70.6	186.3	5941
Monthly average	c	Wf	0.5	26.5	14	0.5	1.5	40.25	14.5	6.75	28	102.75	18.5	12.5	13.25	279.5
		Sh	0.16	31.65	32.14	0.98	3.15	89.3	20.8	4.65	54.14	359.81	12.31	14.64	6.47	630.2
	v	Wf	4	69.5	14.75	1.25	0.25	10.75	20.5	12.5	52	136	18.75	16.75	7	364
		Sh	0.16	174.25	19.07	0.87	3	92.15	32.61	10.86	67.45	291.79	18.29	8.82	23.29	742.61

1977–1979

			Di	Co	O	Th	Het	Hom	Col	Lep	Dipt	Hym	Aran	Acari	Others	Total
1977–1979	c	Wf	72	3034	268	90	146	2046	986	274	2484	2638	422	1488	818	14766
		Sh	21.2	3461.3	483.1	45.5	129.9	6394.4	916.7	134.7	2328.4	5464.6	312.2	1930.8	1562.7	23185.5
	v	Wf	54	2028	198	30	46	512	810	156	2024	2618	322	680	520	9998
		Sh	37.2	4698.7	340.8	45.3	95.2	1788.8	926.3	216.8	2188.8	4900.2	391.8	1119.6	505	17254.5
Monthly average	c	Wf	3.13	131.91	11.65	3.91	6.35	88.95	42.87	11.91	108	114.69	18.35	64.69	35.56	641.97
		Sh	0.92	150.49	21	1.98	5.65	278.02	39.86	5.85	101.23	237.59	13.57	83.95	67.94	1008.05
	v	Wf	3.6	135.2	13.2	2	3.06	34.13	54	10.4	134.93	174.53	21.47	45.33	34.67	666.52
		Sh	1.62	204.29	14.82	1.97	4.14	77.77	40.27	9.42	95.16	213.05	17.03	48.68	21.96	750.18

The seasonal dynamics of the main groups

Although the mosaic-complex character of the area could allow to discuss the seasonal dynamics according to space levels respectively, we don't aim to consider it in the present paper, as above we have mentioned the significance of the space levels in the development of the groups, and we saw the difference in the amount of insects growing by unit areas. On the other hand in connection with the group approach we can suppose that although the levels harshly differ as regards the number of animals growing there, probably these animals do not spend all their life on the same level, i.e. they can make use of either the sand dune or the wind-furrow. This explains our interaction to examine the area as a whole.

Among the phytophages the Orthoptera are salient as regards their significance. It is the first herbivorous group because of the biomass, if not because of its number of entities (Table IV), (GALLÉ et al., 1981). Its seasonal dynamics shows a high peak, which is always in early summer in each of the 4 years (Fig. 5). It is because of the larvae growing mainly then. They proliferate mainly in rainless, dry years; the density can be 2–3 times higher than that of the rainy years' data. After the May–June peak their number decreases rapidly.

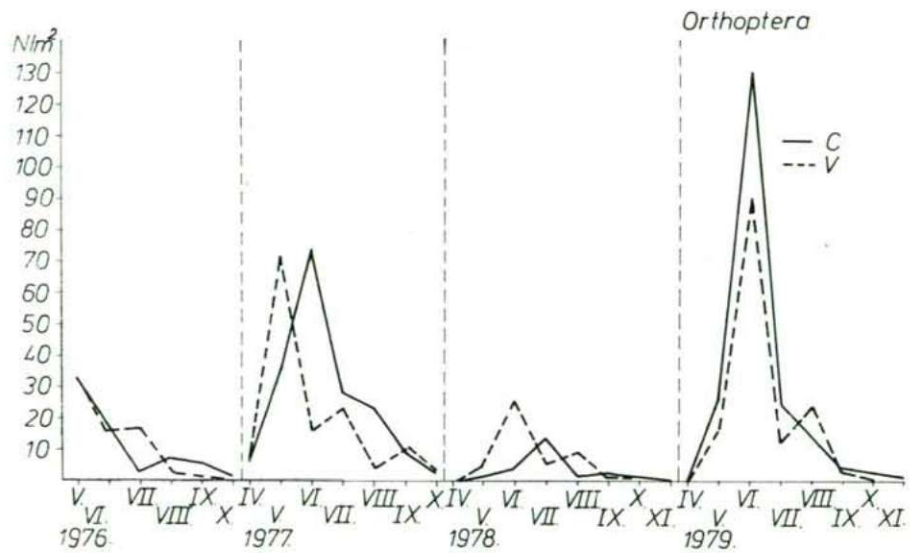

Fig. 5. Seasonal dynamics of Orthoptera.

Among the phytophages the Homoptera order has the biggest number of entities. Its seasonal dynamics is not so definite as the Orthoptera's, because this order is rather homogeneous. Although the steady and numerous presence of the aphids is not characteristic for the area, they cause the high number of specimens under the constant traps. It is clear, that although aphids can grow in the area, their majority leaves this living place (Fig. 6). The data of the changing traps contain mainly the members of the Cicadina suborder. In every year a two-peaked curve can

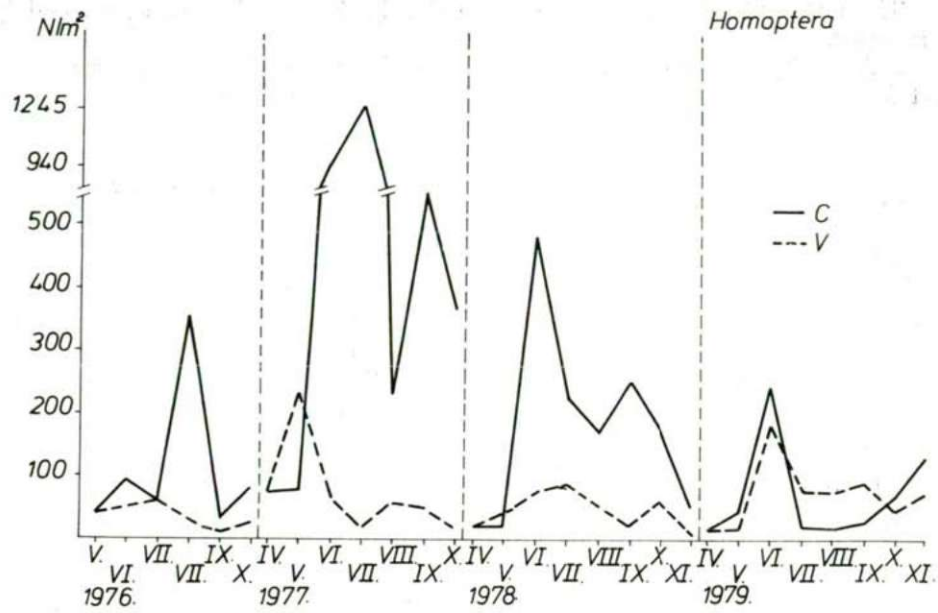

Fig. 6. Seasonal dynamics of Homoptera.

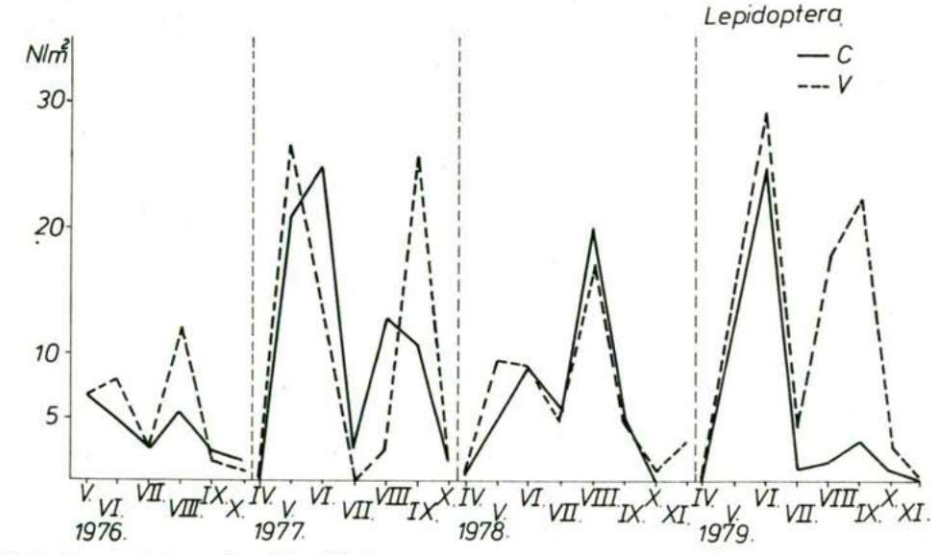

Fig. 7. Seasonal dynamics of Lepidoptera.

be observed. The first, generally the higher peak falls on the late spring, early summer period, and the second one falls on late summer.

We can also find a rather definite dynamism in the case of the Lepidoptera in the examined years. Its peak of flight is in May–June and August–September (Fig. 7). The density of individuals was almost the same during the 3 years.

The Heteroptera's density is similar (Fig. 8). The aphids are active mainly in summer.

Neither the number of the Heteroptera nor that of the Thysanoptera is so high that definite trends could be separated (Fig 9).

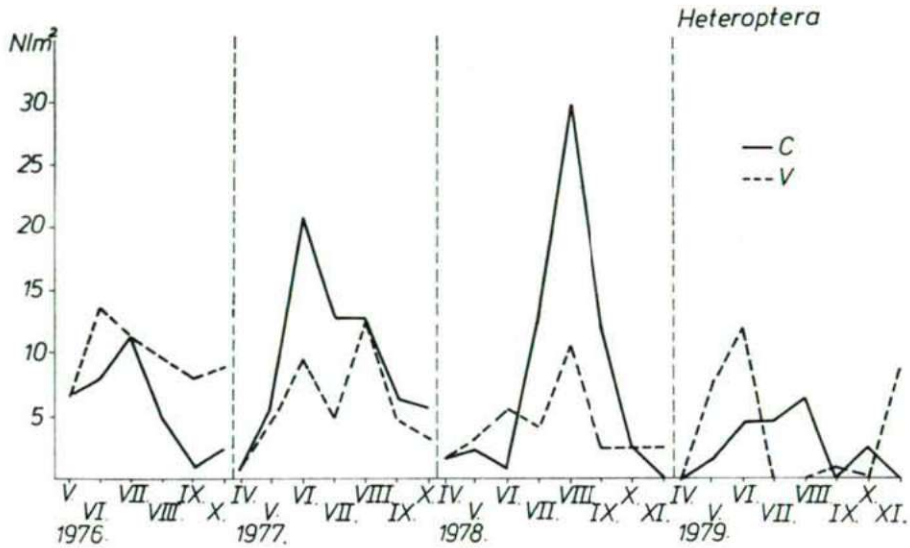

Fig. 8. Seasonal dynamics of Heteroptera.

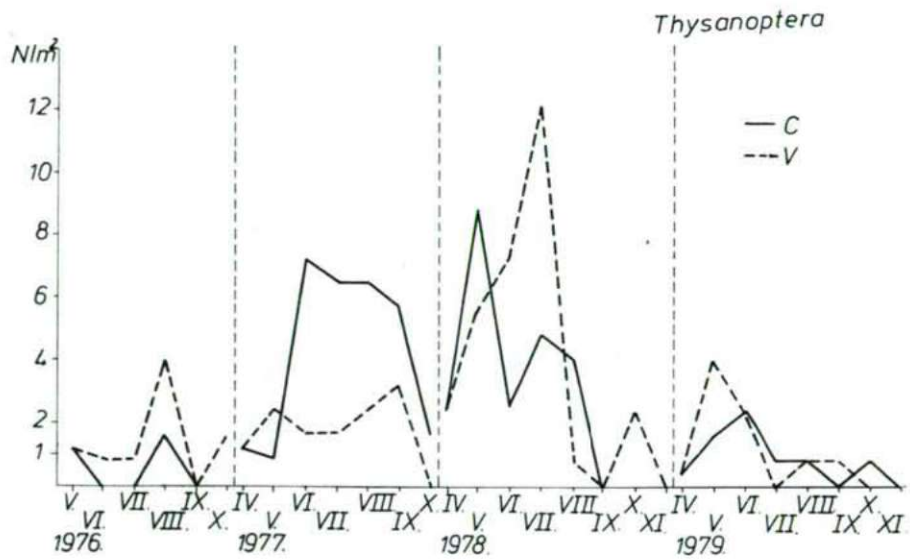

Fig. 9. Seasonal dynamics of Thysanoptera.

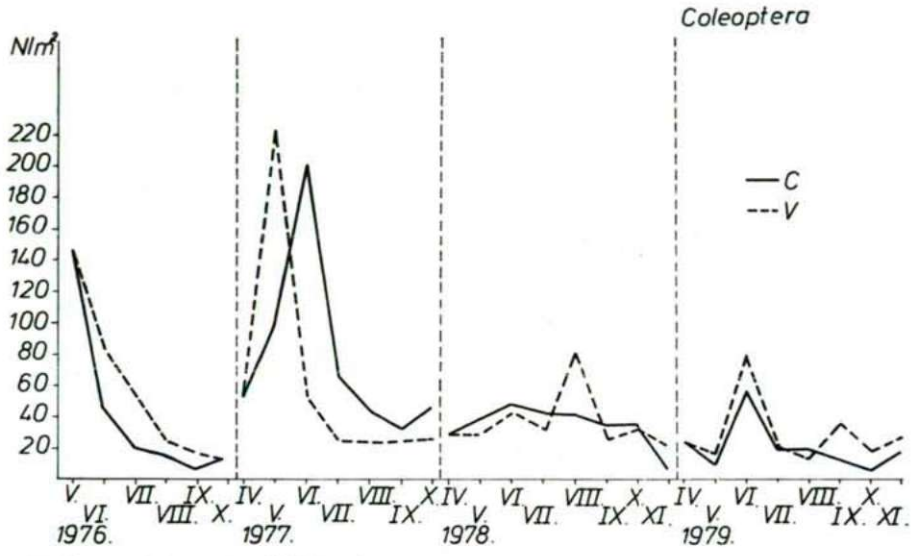

Fig. 10. Seasonal dynamics of Coleoptera.

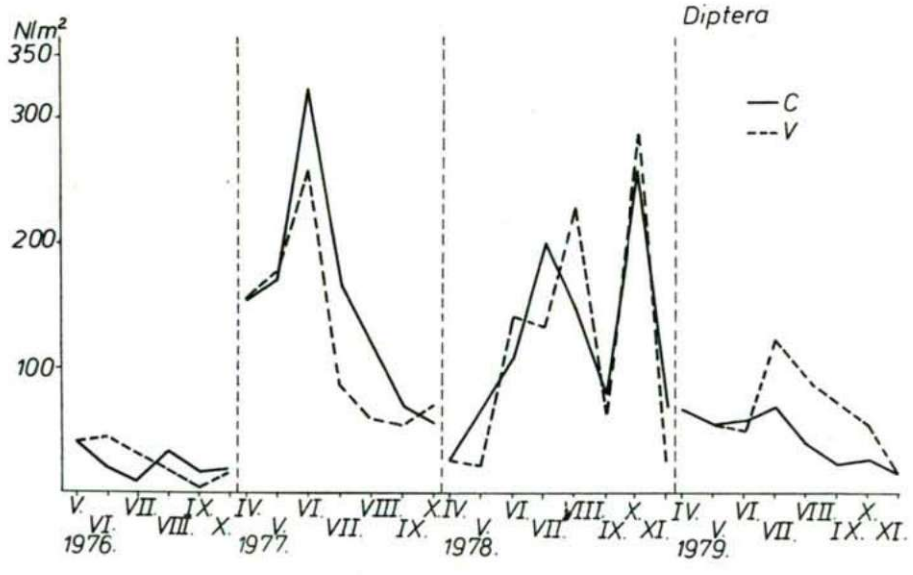

Fig. 11. Seasonal dynamics of Diptera.

The amount of the Coleoptera was more significant in the first two years, but the density in 1978–1979 decreased to half of the original amount (Fig. 10). The most possible reason for this is that after grazing the Scarabaeidae species gradually disappears from the area. That's why the previous definite peak of the number of individuals became indistinct or shifted to summer (1978) or changed into a curve with two peaks (1979).

The Diptera order — the number of species of which is rather high-is represented by very heterogeneous populations as regards nutrition biology. This might cause the group's different behaviour considering seasonal dynamics (Fig. 11). Its number of individuals generally reaches the peak in summer (June–July), but in 1972 there was a second peak in late autumn. The reason for the low density in 1976 might be the fact that the area freed from graze had not reached the appeal of the next years, more characteristical phytocenosis that ensured the mobile Diptera populations of the surrounding areas.

On the other hand the more favourable possibilities of development that appeared in 1976 have made their effect felt only from the next years. It seems that the boom of the start develops towards a state of balance, and in 1979 it reaches the double of the density of the first year. The accurate examination of all these is certainly possible only after the classification to the species and the evaluation of populations respectively.

The seasonal dynamics of the Hymenoptera is determined by the Formicoidea (Fig. 12). Its peak is in June–August. The other Hymenoptera — which are the 0.1–0.2 part of the whole — do not show such great oscillation. Generally there is a June and a September–October peak in the number of individuals, but there was no summer decline in 1978, because this year had a steady climate, and the peak lasted from July to September.

The seasonal dynamics of the spiders (Fig. 13) was rather various during the 4 years. Generally we found the minimum of density in the warmest months, if followed by draught. BALOGH and LOKSA (1948) also found the minimum in August in the Festucetum vaginatae association in the case of two dominant species, while

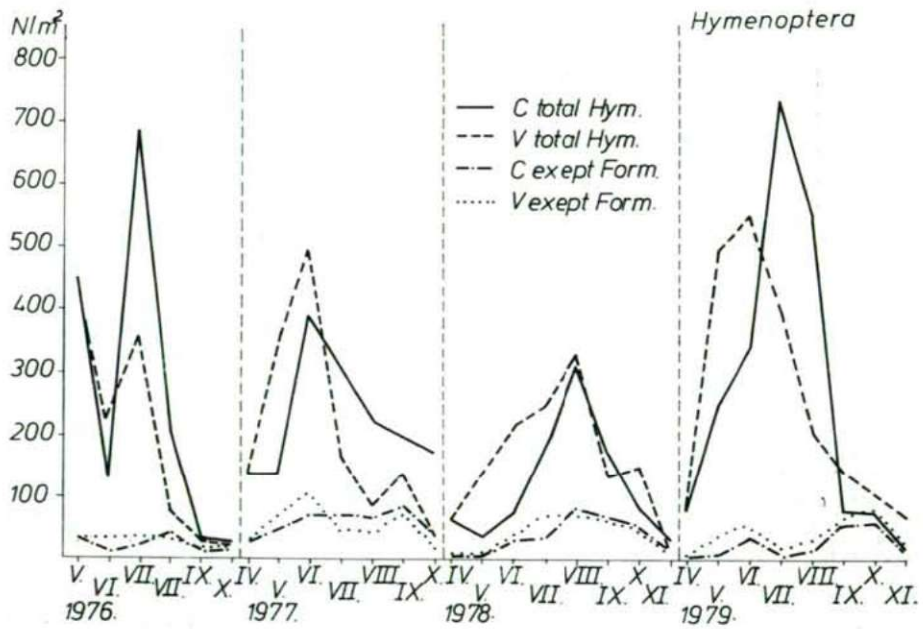

Fig. 12. Seasonal dynamics of Hymenoptera.

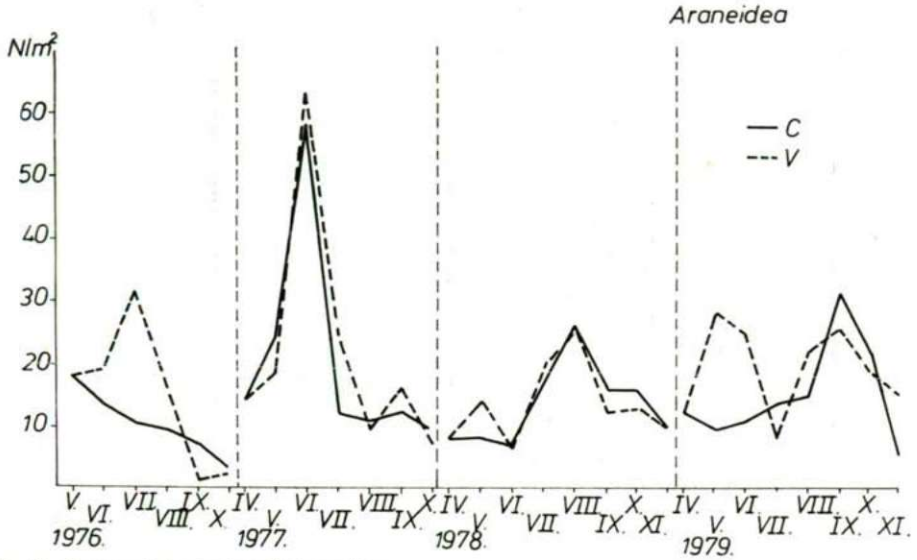

Fig. 13. Seasonal dynamics of Araneidea.

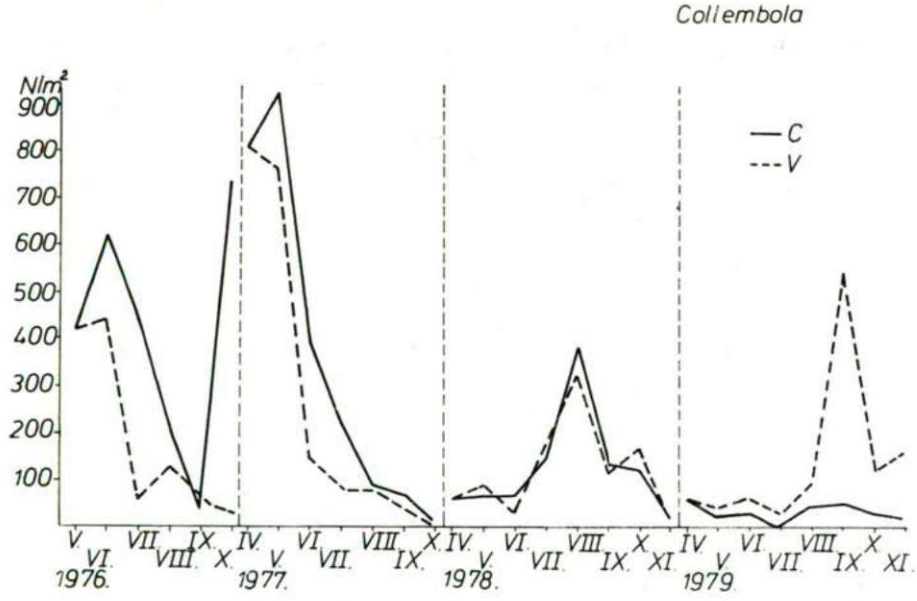

Fig. 14. Seasonal dynamics of Collembola.

FARKAS stated (1978) that the seasonal dynamics shows a saturation curve with a small summer decline.

The fewest of the decomposer Collembola got to the traps in the warm summer months (Fig. 14). In 1978 however it reached an August peak because of the rainy

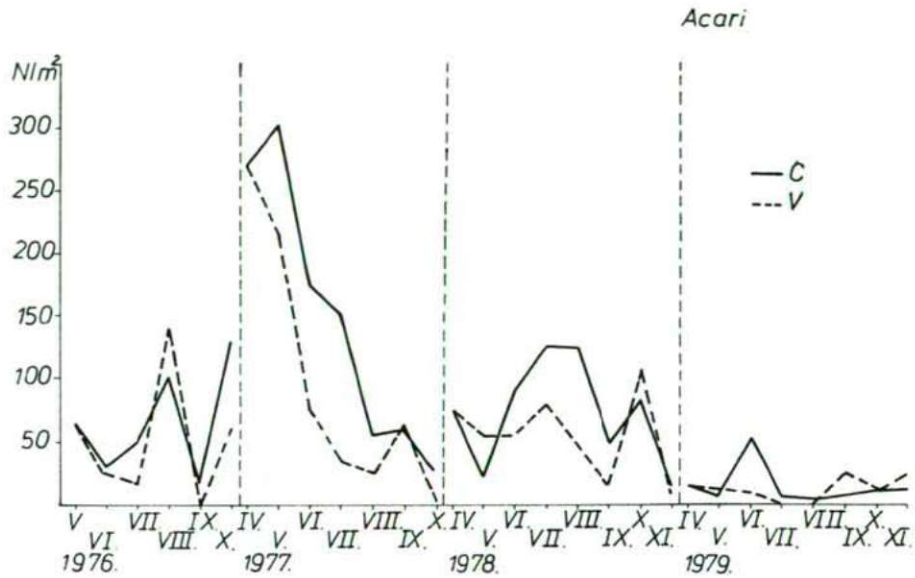

Fig. 15. Seasonal dynamics of Acari.

weather. It is very difficult to find seasonal trends in the behaviour of the group because it may completely differ in the case of each species.

In the case of the Acari a definite, regular dynamics cannot be found (Fig. 15). The density of 1977 — about 300 p/m² — was decreasing rapidly till 1979, so the significance of the Acari — at least that of those, which move in the vegetation and on the soil surface — continuously declines.

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ON THE HIST ORICAL ANTHROPOLOGICAL COLLECTION OF THE ATTILA JÓZSEF UNIVERSITY

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Abstract

The author gives a survey of the historical anthropological collection of the Department of Anthropology of the University in Szeged. Tables present the findings distributed according to sexes, from different findspots enumerating the authors of different series.

He mentions that the collection is based on 8700 findings, completed by further some 2000 findings, earlier stored in Budapest, in the Anthropological Collection of the Museum of Natural Sciences. Further additional 2000 findings are at present under registration.

The findings have so far been evaluated primarily from taxonomical point of view. At present, mainly the scientific evaluation of the palaeopathological deformations has come into spotlight.

As to the size of the historical anthropological collection, it is to be considered as the 5th–6th largest collection in Europe.

Key words: historical anthropology, collection.

The Department of Anthropology was established at the University of Szeged in 1940. Its first chairholder Professor was Lajos Bartucz till 1959 who studied several fields of the anthropology. In the first third of the century, he published valuable results about the physical development of the Hungarian children, about the characteristics of the adult population — primarily the ethnical groups in the Great Hungarian Plain and Transdanubia — but the main field of his research work was the historical anthropology. Around 1930 the skeletons of several — mainly prehistoric — cemeteries, excavated by FERENC MÓRA in the neighbourhood of Szeged (Southern Hungary) were at last displayed after several removals in the Department of Anthropology of the University in Szeged.

This provided the basis of the collection which has continually increased during the last three decades, first of all as a result of the excavations of the researchers of the Department, and those of the archaeologists of the museums of Szeged and of the South of the Hungarian Great Plain (OTTÓ TROGMAYER, MIHÁLY KŐHEGYI, KATALIN NAGY, ELVIRA H. TÓTH etc.).

On the collection according to archaeological periods a survey was published by ANTÓNIA MARCSIK in 1977 (MARCSIK, 1977). Table 1 shows the present-day situation.

It turns out that the oldest skeletons of the collection derive from the neolithic period, from the beginning of which, the Department has anthropological findings practically from each period.

The collection consists at present 8700 findings the distribution of which according to archaeological periods is: prehistoric period 1159, Migration period 4251, Hungarian conquest 278, Arpadian-age 1834, Middle-Ages 1045, while the findspot

Table 1. The distribution of historical anthropological collection of the University Szeged according to the archaeological periods

Archaeological Ages	The number of finds	%	The number of findspots	%
Neolithic	71	0.82	21	5.4
Copper Age	127	1.46	16	4.1
Bronze Age	885	10.17	23	5.9
Iron Age	76	0.76	10	2.6
Sarmatian Age	487	5.60	46	11.8
4th–5-th Centuries	297	3.41	19	4.9
Avarian period	3467	39.85	101	25.9
10th Century	278	3.20	38	9.7
10th–11th Centuries	258	2.97	12	3.1
Arpadian Age	1576	18.11	29	7.4
Middle Ages	1045	12.01	43	11.0
Unknown	133	1.53	32	8.2
Together:	8700	100.00	390	100.00

of 133 finds is unknown. These figures, however, do not contain the total number of the skeletons accumulated at the Department, as the findings of the recent excavations (another some 2000 skeletons) are being or will be cleaned and registrated.

This collection was completed, by acquiring some further 2000 findings in the last decade having been stored in Budapest, in the Anthropological Collection of the Museum of Natural Sciences.

So the historical anthropological collection consists, therefore, of more than 12 thousand findings. As a matter of fact, it might take the 5th–6th place among the most considerable collections in Europe.

The number of skeletons from the Avar period (39.9 p.c) is considerable but the collection contains numerous skeletons from the Arpadian age as well (in Hungarian history: 897–1301: 18.1 p.c), the Middle Ages (12.0 p.c), and the Bronze Age (10.2 p.c).

390 of the 8700 findings derive from findspots in Southern Hungary. But not all the skeletons excavated in the mentioned area, were enumerated into the collection. Findings from the Avar period (25.9 p.c), as well as those from the Sarmatian age (11.8 p.c) and from the Middle Ages (11.0 p.c) are represented in the highest ratio according to findspots.

As has already been mentioned Professor LAJOS BARTUCZ began the scientific survey of findings, who published more than 250 scientific papers; several of them on historical anthropology. From 1960 till 1980, the head of the Department was PÁL LIPTÁK, who similarly studied historical anthropology. His interest was primarily directed to the findings of the Avar period and of the age of Hungarian conquest (last years of the 9th century), as well as to the problems of the Hungarian prehistory. His co-workers — the author of this paper — as well as his students (ANTÓNIA MARCSIK, EDITH LOTTERHOF, KÁROLY VÁMOS, IMRE VARGA) also participated in surveying. Owing to this the description of 4204 of the 8700 skeletons has been published in detail.

The condition of the findings is very different. That of those from the prehistory may be considered good. On the other hand — due to the way of excavation in the first third of our century (rescuing only the well preserved adult crania)

— that's why the anthropological series of cemeteries aren't complete and consequently, practically unsuitable for drawing paleodemographical conclusions. At the same time, the prehistoric findings originating from recent excavations, like for instance those from Tápé, are fragmentary.

The state of preservation of the findings from the Sarmatian age may be considered the worst. This must be due to the disarrangement of these graves soon after the burial. Fortunately, however, the Gepid, Avar skeletons and those from Hungarian conquest and particularly from the Arpadian age — excavated mostly in the

Table 2. Finds published in detail. — Prehistoric Age.

Findspots	Archaeological Ages	The name of author, the year of publication	Males	Females	Juvenile	Unknown
Békés–Povádzug	Neolithic	Lipták–Farkas, 1967	4	2	—	—
Deszk	Neolithic	Lipták, 1974/75	—	2	—	—
*Vajška–Baba–Sivac	Late Neolithic	Farkas, 1973	1	3	—	—
Vésztő–Mágori mound	Neolithic	Farkas, 1974	4	3	—	—
Balmazújváros–Árkusmajor	Copper Age (tumulus)	Marcsik, 1979	1	—	—	—
Csongrád–Kettőshalom–Bárdos farm	Copper Age	Marcsik, 1971/72	1	—	—	—
Debrecen–Basahalom	Copper Age (tumulus)	Marcsik, 1979	2	—	—	—
Debrecen–Dunahalom	Copper Age (tumulus)	Marcsik, 1979	1	—	—	—
Debrecen–Halászlaponyag	Copper Age (tumulus)	Marcsik, 1979	—	1	—	—
Dévaványa–Barcéhalom	Copper Age (tumulus)	Marcsik, 1979	1	—	—	—
Dévaványa–Csordajárás	Copper Age (tumulus)	Marcsik, 1979	—	—	3	—
Kétegyháza	Copper Age (tumulus)	Marcsik, 1979	3	2	3	—
Magyarhomoróg–Kónyadomb	Copper Age	Farkas, 1976	17	15	7	9
*Nosza–Gyöngypart	Copper Age	Farkas, 1973	6	2	—	—
Sárrétudvari–Balázshalom	Copper Age (tumulus)	Marcsik, 1979	—	1	—	—
Battonya	Early Bronze Age	Farkas–Lipták, 1968	11	13	25	10
*Mokrin	Early Bronze Age	Farkas–Lipták, 1972 Lengyel–Farkas, 1972	82	92	61	2
Pitvaros	Early Bronze Age	Farkas, 1971	6	7	1	—
Tápé Charcoal-burning site	Late Bronze Age	Farkas–Lipták, 1971 Farkas–Lipták, 1975	186	159	162	72
*Gomolava	Iron Age	Farkas–Marcsik, 1976	7	18	51	—
		Together:	333	320	313	93

* Finds from Voivodship (North-Jugoslavia)

Table 3. Finds published in detail. — Migration Period.

Findspots	Archaeological Ages	The name of author, the year of publication	Males	Females	Juvenile	Unknown
Hódmezővásárhely–Fehértó	Sarmatian Age	BARTUCZ, 1961	8	8	5	—
Madaras–Hillocks	Sarmatian Age	MARCSIK, 1974	—	1	—	—
Mélykút–Sáncdűlő	Sarmatian Age	FARKAS–LENGYEL–MARCSIK, 1971	—	1	—	—
Püspökladány–Görepart	Sarmatian Age	MARCSIK, 1974	3	2	1	—
Szentes–Kistőke	Sarmatian Age	BARTUCZ, 1961	5	4	1	—
Kiszombor	Gepid	BARTUCZ, 1936 LIPTÁK–MARCSIK, 1977	38	18	6	1
*Subotica	Migration Period	FARKAS, 1973	—	1	—	—
*Ada	Migration Period	FARKAS, 1973	1	—	—	—
Biharkeresztes–Ártánd	4th–5th Centuries	LIPTÁK–MARCSIK, 1977	4	5	1	—
*Adorján	Avarian Period	BARTUCZ–FARKAS, 1957	50	35	14	—
*Bačka-Topola	Avarian Period	FARKAS, 1973	1	1	—	—
Biharkeresztes–Toldi lane	5th–6th Centuries	LIPTÁK–MARCSIK, 1977	3	4	—	—
Fehértó–A.	Avarian Period	LIPTÁK–VÁMOS, 1969	89	88	25	2
Kunszállás	Avarian Period	LIPTÁK–VARGA, 1971/72	11	17	22	—
Madaras–Briek–Works	Avarian Period	LIPTÁK–MTCSIK 1976	24	34	30	—
Mélykút–Sáncdűlő	Avarian Period	FARKAS–LENGYEL–MARCSIK, 1971 MARCSIK, 1971	15	22	12	—
Rákóczifalva–Kastélydomb	Avarian Period	LIPTÁK–MARCSIK, 1975	18	17	8	—
Sükösd–Ságod	Avarian Period	KŐHEGYI-MARCSIK, 1971	41	68	56	—
Szabadszállás–Boczka farm	Avarian Period	LIPTÁK, 1972	1	—	—	—
Szarvas–Kákapuszta–Double-hillock	Avarian Period	LIPTÁK–MARCSIK, 1970/71	16	9	11	—
Szeged–Kundomb	Avarian Period	LIPTÁK–MARCSIK, 1966	62	76	33	5
Szeged–Makkoserdő	Avarian Period	VÁMOS, 1973	57	45	41	9
Szentes–Kaján	Avarian Period	BARTUCZ, 1958	43	26	—	—
Szekszárd–Palánkpuszta	Avarian Period	LIPTÁK, 1974	48	55	27	6
		Together:	538	537	293	24

* Finds from Voivodship (North-Jugoslavia).

recent decades by archaeologists of Museums and partly by those of our Department — have remained in a very good state of preservation.

The prehistoric findings of the collection — with the exception of the insignificant number of skeletons deriving from recent excavations — are described in the framework of a larger work. Some details and the main experiences of this have already been published in various journals. The findings from findpots in Northern Jugo-

slavia, like those from Vajška, Nosza, Mokrin, Gomolava, are also connected with these. The sexual distribution of the skeletons of the studied prehistorical findings and findspots is shown in Table 2.

The number of the published findings on the Migration period is also very considerable (1392), dating back primarily to the Sarmatian age and Avar period (Table 3).

128 skeletons from the Hungarian conquest are similarly published (Table 4), as well as 1779 finds from the Arpadian age and the Middle Ages (Table 5).

Till 1960 the evaluation of findings had been sporadically performed — but after that consequently — according to similar metric and morphological programmes, in order to facilitate comparisons. In the last twenty years, the evaluation of findings was performed on the basis of a taxonomical analysis, elaborated by LIPTÁK and applied to the historical material.

In the latest decade palaeopathologic analyses were carried out, restricted, at present, to the findings from the Avar period. These investigations began, however, much earlier because the posthumous work of LAJOS BARTUCZ (BARTUCZ, 1966)

Table 4. Finds published in detail. — Hungarian Conquest.

Findspots	Archaeological Ages	The name of author, the year of publication	Males	Females	Juvenile	Unknown
Békés–Povádzug	Hungarian Conquest	LIPTÁK–FARKAS, 1967	2	—	1	—
Gerendás–Petőfi Co-operative farm	Hungarian Conquest	MARCSIK, 1974	1	1	—	—
Harta–Béke Co-operative farm	Hungarian Conquest	MARCSIK, 1974	1	—	1	—
Hódmezővásárhely–Nagysziget	Hungarian Conquest	FARKAS–LOTTERHOF–MARCSIK, 1969	5	6	4	—
Izsák–Balázspuszta	Hungarian Conquest	MARCSIK, 1976	1	—	—	—
Kecskemét–Városföld	Hungarian Conquest	MARCSIK, 1974	1	3	1	—
Kübekháza–Újtelep	Hungarian Conquest	FARKAS–LOTTERHOF–MARCSIK, 1969	7	4	2	—
Rákóczifalva–Kastélydomb	10th Century	LIPTÁK–MARCSIK, 1975	—	2	—	—
Szabadkígyós–Modelfarm	Hungarian Conquest	LOTTERHOF, 1971	12	9	6	—
Szabadkígyós–Pál Coppice	Hungarian Conquest	LOTTERHOF, 1971	10	7	1	—
Szalkszentmárton–Paréjoshát	Hungarian Conquest	MARCSIK, 1974	1	1	8	—
Szarvas–Tessedik street	10th Century	LIPTÁK–MARCSIK, 1971	2	3	2	
Öttevény	Hungarian Conquest	LIPTÁK, 1962	1	—	—	—
Aldebrő–Mocsáros	10th–11th Centuries	Marcsik, 1967	10	7	5	—
		Together:	54	43	31	—

had already analysed in detail the trephined findings, stored not only in the Szeged collection but also those being in other Hungarian collections.

It is to be mentioned here that in the Szeged collection there were 30 distorted (macrocephalous) crania and several trephined findings. These, evaluated in Lajos BARTUCZ's palaeopathological work, as well, are at present in unknown place. The number both of macrocephalous and trephined findings is, therefore, small in our Department at present.

We do not intend to speak about the results of the research work in detail, as they have been published in a great number of publications. In the Tables findspots, the date of publishing, the findings and the names of the authors are also given. So bibliographical works given in the list of references may informe everybody about the other data of the publication. These articles were mainly published in the annuai publications of the biologists of the University in Szeged (Acta Biologica Szegedien-

Table 5. Finds published in detail. — Arpadian Ages and Middle Ages.

Findspots	Archaeological Ages	The name of author, the year of publication	Males	Females	Juvenile	Unknown
Békés–Povádzug	Arpadian Age	LIPTÁK–FARKAS, 1967	31	41	72	—
Csongrád–Felgyő	Arpadian Age	BARTUCZ–FARKAS, 1956	17	16	5	—
Kardoskút–Fehértó	11th–12th Centuries	MARCSIK, 1970	136	119	41	—
Nagybaracska–Öregszőlők	11th Century	MARCSIK, 1972	6	3	5	—
Nádudvar–Töröklaponyag	10th–11th Centuries	LIPTÁK, 1968	14	15	9	—
Orosháza–Rókóczi settlement	10th–13th Centuries	FARKAS–DEZSŐ, 1955	40	29	—	—
Orosháza–Rákóczi settlement	Arpadian Age	LIPTÁK–FARKAS, 1962	93	78	27	4
Szarvas–Őszőlő	10th–11th Centuries	LIPTÁK–MARCSIK, 1971	6	2	2	1
Szatymaz–Railway station	10th–12th Centuries	LIPTÁK–FARKAS, 1967	114	89	83	—
*Zenta–Farkas farm	Arpadian Age	BARTUCZ–FARKAS, 1958	9	5	4	—
*Zenta–Paphalom	Arpadian Age	BARTUCZ–FARKAS, 1958	68	42	36	1
Baja–Pető	11th–16th Centuries	LOTTERHOF, 1968	74	58	62	15
*Ludoš–Csurgó	15th Century	FARKAS, 1971	1	—	—	—
Nagybaracska–Kisbaracska	14th–16th Centuries	MARCSIK, 1972	1	2	1	—
Nagybaracska–Piszkula	14th–16th Centuries	MARCSIK, 1972	1	—	3	—
Röszke–Kószó farm	14th–15th Centuries	LOTTERHOF, 1971	21	19	27	—
Téglás–Angolkert	11th–14th Centuries	LIPTÁK–MARCSIK, 1965	15	21	17	—
*Zombor–Airport	15th–17th Centuries	BARTUCZ, 1960	87	38	52	—
		Together:	734	577	446	22

* Finds from Voivodship (North-Jugoslavia).

sis), as well as in the central journal of the Hungarian anthropology (Anthropological Közlemények — Anthropological Publications), but in addition to these, some analysis of the findings in the collection in Szeged may also be found in the annual publications of some Museums (Cumania, Yearbook of the Museum in Szeged, Yearbook of the Déri Museum in Debrecen), as well as in foreign journals, an independent bibliography informs about the publications till 1952 (ALLODIATORIS, 1958) and about those after 1952 the Anthropological Publications, published usually in every two year (FARKAS and DEZSŐ, 1965; FARKAS, 1966, 1968, 1969, 1972, 1973; FARKAS and MARCSIK 1976, 1978; FARKAS, KURCSIK, and MARCSIK 1981).

The storing technique of the collection (the post-cranali skeletons are on shelves with metal frames in wooden cases, the skulls in paper boxes within cases), can be considered as good. The size of the store and the storing conditions are, however, far from being satisfactory (the skeletons are mostly in cellars). The possibilities of further development are, unfortunately, very limited, because our Department deals primarily with education. We are convinced nevertheless, about being obliged to preserve the anthropological findings of our collection excavated from the Hungarian earth and to bring within reach for the scientific life the findings still not published.

Present work is particularly supported by the Hungarian and foreign research workers wo have already analysed these findings, according to some point of view, respectively participating in the processing of the material of the collection. From among the Hungarian dentists, we may mention here the names of GYÖRGY HUSZÁR, KÁROLY TÓTH, GÁBOR KOCSIS; from among those participating in the palaeopathological research ELEMÉR ANTAL, FERENC KÓSA; from among the foreigners MICHAEL FINNEGAN, NIKOLAOS XIROTIRIS. Having provided the metric data of the prehistoric findings to the data-bank in Mainz we hope to attribute to the work of the investigators of prehistory.

In the future, we want to continue the historical anthropological research work, though — owing to the personal change in our staff — we should like to concentrate the profile of research on investigating of the living population. But we aim to publish the analyse of our larger collections according to archaeological periods, and offer our support in this respect to Hungarian and foreign colleagues.

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ON THE PUBERTY OF GIRLS IN SZEGED, HUNGARY

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Abstract

The physical development of 6–18 years old children of Szeged (Southern Hungary) was recorded with sampling in 1958/59, in 1961 and in 1966/67. In the spring of 1981, as a continuation of this work, the authors collected data concerning the puberty of schoolgirls aged 10–14 of the town, which has a population of about 200.000 inhabitants. Their experiences about the physical development of 1099 schoolgirls are summarized in this study. Their present results were compared with previous samplings. The samples ensue on single samplings each. It has been found, that in the case of girls, with the same age-groups, the arithmetic mean of body height and body weight, has increased in every sample in comparison to the preceding samples. The mean of the normal circumference of chest has decreased in the latest sample, and the median of the menarche has also decreased in every sample. Relying upon these findings, it is supposed, that the course of the secular trend continues even now, in girls of Szeged.

The authors point out, that there is not a definite body height or body weight deciding the physiological maturity. They are planning to examine about 20.000 pupils in the frame of the research work, which has been started in 1981.

Introduction

It is obvious that the adolescence is one of the most problematical, but at the same time, one of the most beautiful period of human life. Learning about this topic is equally the task of the research workers, educators and parents. Fortunately we can consider this question from each aspect. On the present occasion, we should like to publish the results of an anthropological examination on female pupils of a Southern Hungarian city, mainly from the aspect of the research worker.

In Szeged up to the present four examinations were carried out; in 1958/59, in 1961, in 1966/67 and in spring of 1981, to state the varieties of boys and girls according to age-groups. We have reported about our previous samplings in numerous publications (FARKAS, 1961; 1962; 1963; 1964; 1966; 1967; 1969; 1970; 1972), mentioning about the establisment of the time of menarche, besides the growth examinations. Recently we have continued the research of menarche and examined relationships between the age of puberty and the natural and social factors. About this has also been reported in several articles of scientific periodicals (FARKAS, 1979; 1980).

Table 1. The parameters of body height of 11 to 14.5 years old girls in Szeged.—1981

Menstruated				Age (years)	Not menstruated				Together				Age (years)	$\bar{X}-\bar{x}_1$	$\bar{X}-\bar{x}_2$
n_1	$\bar{x}_1$	s_1	w		n_2	$\bar{x}_2$	s_2	w	n_1+n_2	$\bar{X}$	s	w			
9	149.34	8.80	132.0–161.6	11	146	144.02	6.70	128.2–159.8	155	144.33	6.95	128.2–161.6	11	−5.01	0.31
17	156.32	7.20	145.7–169.1	11.5	136	146.70	7.11	128.9–161.1	153	147.77	7.73	128.9–169.1	11.5	−8.55	1.07
54	155.23	9.88	140.1–172.5	12	107	149.01	7.46	127.8–164.6	161	151.10	8.85	127.8–172.5	12	−4.13	2.09
58	157.33	5.23	144.6–168.7	12.5	92	151.13	6.30	134.9–165.0	150	153.53	6.64	134.9–168.7	12.5	−3.80	2.40
97	158.52	6.07	143.7–180.1	13	75	154.17	6.41	141.6–170.1	172	156.63	6.58	141.6–180.1	13	−1.89	2.46
108	158.53	8.08	147.9–173.1	13.5	29	154.46	9.39	126.7–173.3	137	157.67	8.54	126.7–173.3	13.5	−0.86	3.21
100	160.32	5.37	148.7–174.2	14	17	158.14	6.33	147.0–170.2	117	160.00	5.57	147.0–174.3	14	−0.32	1.86
50	159.26	6.42	144.5–170.1	14.5	4	157.50	6.83	152.4–167.5	54	159.13	6.47	144.5–170.1	14.5	−0.13	1.63
493					606				1099						

Table 2. The parameters of the body weight of the 11 to 14.5 years old girls in Szeged. — 1981

Menstruated				Age (years)	Not menstruated				Together				Age (years)		
n_1	$\bar{x}_1$	s_1	w		n_2	$\bar{x}_2$	s_2	w	n_1+n_2	$\bar{X}$	s	w		$\bar{X}-\bar{x}_1$	$\bar{X}-\bar{x}_2$
9	44.44	12.01	35.7–68.5	11	146	37.10	9.41	23.1–82.9	155	37.53	9.82	23.1–82.9	11	−8.91	0.43
17	45.47	6.91	37.2–63.0	11.5	136	37.57	8.44	23.7–70.7	153	38.45	8.64	23.7–70.7	11.5	−7.02	0.88
54	49.23	9.25	31.2–68.9	12	107	39.45	7.79	28.2–63.3	161	42.73	9.50	28.2–68.9	12	−6.50	3.28
58	49.43	8.75	36.8–84.8	12.5	92	41.50	8.43	24.3–66.9	150	44.57	9.38	24.3–84.8	12.5	−4.86	3.07
97	50.93	8.68	37.7–90.3	13	75	44.16	10.19	31.9–80.9	172	47.98	9.95	31.9–90.3	13	−2.95	3.82
108	50.06	9.79	34.0–81.0	13.5	29	43.86	11.30	39.5–87.2	137	48.75	10.43	34.0–87.2	13.5	−1.31	4.89
100	51.62	8.74	40.0–84.7	14	17	50.43	7.52	40.0–67.9	117	51.45	8.58	40.0–67.9	14	−0.17	1.02
50	53.00	10.67	40.0–88.9	14.5	4	43.33	8.50	31.8–52.2	54	52.28	10.82	31.8–88.9	14.5	−0.72	8.95
493					606				1099						

Table 3. The parameters of normal chest circumference of 11 to 14.5 years old girls in Szeged. — 1981

Menstruated				Age (years)	Not menstruated				Together				Age (years)	$\bar{X}-\bar{x}_1$	$\bar{X}-\bar{x}_2$
n_1	$\bar{x}_1$	s_1	w		n_2	$\bar{x}_2$	s_2	w	n_1+n_2	$\bar{X}$	s	w			
9	74.11	9.84	64–95	11	146	68.68	8.42	56–103	155	69.00	8.60	56–103	11	−5.11	0.32
17	74.35	5.06	62–85	11.5	136	68.65	7.49	56–94	153	69.28	7.48	56–94	11.5	−5.07	0.63
54	79.20	7.16	63–95	12	107	70.63	6.55	59–93	161	73.50	7.88	59–95	12	−5.70	2.87
58	79.34	6.88	68–99	12.5	92	72.71	7.30	57–101	150	75.25	7.84	57–101	12.5	−4.09	2.54
97	80.75	7.36	69–108	13	75	74.31	8.42	63–102	172	77.94	8.46	63–108	13	−2.81	3.63
108	79.52	8.80	61–107	13.5	29	74.72	8.86	63–106	137	78.50	9.02	61–107	13.5	−1.02	3.78
100	80.98	7.00	67–105	14	17	79.94	6.39	71–93	117	80.83	6.92	67–105	14	−0.15	0.89
50	82.58	8.89	69–111	14.5	4	69.25	9.18	59–80	54	81.59	9.57	59–111	14.5	−0.99	12.34
492					606				1099						

Table 4. The parameters of bicristal breadth of 11 to 14.5 years old girls in Szeged. — 1981

Menstruated				Age (years)	Not menstruated				Together				Age (years)	$\bar{X}-\bar{x}_1$	$\bar{X}-\bar{x}_2$
n_1	$\bar{x}_1$	s_1	w		n_2	$\bar{x}_2$	s_2	w	n_1+n_2	$\bar{X}$	s	w			
9	24.24	3.00	21.6–31.2	11	146	22.77	2.08	18.0–31.2	155	22.85	2.17	18,0–31.2	11	−1.39	0.08
17	25.08	1.40	22.8–27.7	11.5	136	22.97	1.93	18.2–29.5	153	23.20	1.99	18.2–29.5	11.5	−1.88	0.23
54	25.32	2.00	21.3–30.0	12	107	23.51	1.75	19.2–27.6	161	24.12	2.02	19.2–30.0	12	−1.20	0.61
58	25.69	1.55	22.6–28.8	12.5	92	23.92	1.90	18.8–29.8	150	24.60	1.96	18.8–29.8	12.5	−1.09	0.68
97	25.96	1.68	22.7–32.4	13	75	24.76	1.95	21.8–30.0	172	25.44	1.89	21.8–32.4	13	−0.52	0.68
108	25.72	1.67	21.9–30.5	13.5	29	24.66	2.38	19.1–33.0	137	25.50	1.89	19.1–33.0	13.5	−0.22	0.84
100	25.95	1.80	20.0–32.0	14	17	25.64	1.55	24.0–28.3	117	25.90	1.76	20.0–32.0	14	−0.01	0.26
50	26.71	2.59	21.1–36.7	14.5	4	24.40	2.40	21.3–27.10	54	26.54	2.65	21.1–36.7	14.5	−0.17	2.14
493					606				1099						

Materials and Methods

Latest researches made the authors project to collect data of 20 thousand girls. This work started in February 1981 in Szeged. Untill now we managed to collect the samples of about 3500 adolescent girls, whose age-groups range from 11 to 14.5 years. The research aims an examination into the agents, influencing the appearance of menarche. According to this questions were raised about the family circumstances (e.g. the number of siblings), parents (e.g. their occupation, educational level), and the date of the menarche of the girls. Altogether 34 different informations were collected this way partly from the parents and partly from the pupils. Within the frames of this work has been performed the measuring of body height, body weight, the normal circumference of chest and the bicristal breadth. Due to shortage of both material and personal conditions, we could not establish the similar body measurements of boys, however it should also be necessary, thus we can report here only on our observations dealing with girls alone.

At present, the four body measurements are analyzed in case of 1099 girls of 11 to 14.5 years. The most important parameters are stated (arithmetic mean, standard deviation, range in groups of menstruating and not menstruating girls separately as well as of all girls together (Tables 1–4).

Results

In every age-group was observed that in case of all the four groups, the mean values of the girls, already menstruating, were higher than those of not menstruating (Tables 1–4). Experiments, like this are mentioned by other authors as well (CSÓKA–PHILIPPNÉ JUNG and EIBEN, 1981) in case of Hungarian girls. It is generally mentioned, that the menarche of girls occurs either at reaching a higher arithmetic mean of body weight and stature, or at reaching a certain arithmetic mean of body weight and stature. The works of BODZSÁR (1977), HAMMAR et al. (1972), RICHTER (1973), ROBERTO-GLORIA (1974), WINICK (1975) refer to the first variation. Other authors consider the appearance of menarche to be dependent of 45.8 kg arithmetic mean of body weight, and 153.6 cm arithmetic mean of body height, for Hungarian girls (BORSOS, TAKÁCS and SMID, 1977). HOFMEIER, SCHWIDDER and MÜLLER (1964) connect the menarche with achieving a certain physical development. In Marshall's opinion the menarche appears after achieving a certain height velocity peak.

According to FRISCH (1976) the absolute and relative increase of the adipose tissue can be proved at girls both of early and late maturation. The arithmetic mean of body weight of girls with a median of 14.5 years, was as much as 47.2 kg; but those with 12.4 years showed an arithmetic mean of body weight of 48.7 kg (FRISCH, REVELLE and COOK, 1973). ŠKERLJ, BROŽEK and HUNT (1953) stated, that the median of obese girls was 12.99 years, that of thin ones 13.5 years, whereas of the so-called normaly developed girls was 13.66 years.

So even the thin girls become earlier mature with less body weight, than those who have a higher body weight and are normaly developed physicaly.

KANTERO, WIDHOLM and WIDHOLM (1970) clearly reject the correlation between the stature and the date of appearance of menarche. They mention that the date of the median of menarche diverged at girls whose body weight exceeded, fell behind or neared the arithmetic mean. Girls of lower weight matured later, than those of higher weight. According to our first four tables the data of menstruating girls, namely the arithmetic mean of body weight, the normal circumference of chest, and the bicristal breadth is in every case higher, than at the girls still before menstruation. At the same time, however, it can be stated, that in the case of the body measure-

Table 5. The comparison of arithmetic means of body height, body weight and normal chest circumference at 11 to 14.5 years old girls from Szeged

Age (years)	Body height				Body weight				Normal chest circumference				n	n	n
	I. 1958–59	II. 1966	III. 1981	III.–I.	I. 1958–59	II. 1966	III. 1981	III.–I.	I. 1958–59	II. 1966	III.. 1981	III.—I.	I. 1958–59	II. 1966	III. 1981.
11	139.4	144.0	144.3	4.9	33.9	36.6	37.5	3.6	64.3	71.1	69.0	4.7	74	72	155
11.5	141.7	146.1	147.8	7.1	35.1	38.5	38.5	3.4	65.1	72.4	69.3	4.2	87	113	153
12	145.7	148.4	151.1	5.4	38.1	40.0	42.7	4.6	67.9	74.2	73.5	5.6	75	119	161
12.5	149.0	152.0	153.5	4.5	40.1	44.1	44.6	4.5	68.5	77.6	75.3	6.8	77	112	150
13	152.7	153.7	156.6	3.9	43.5	46.3	48.0	4.5	70.7	80.2	78.0	7.3	69	120	172
13.5	154.3	156.2	157.7	3.4	46.0	49.0	48.8	2.8	74.4	81.2	78.5	4.1	68	89	137
14	156.9	156.1	160.0	3.1	47.6	49.8	51.5	3.9	76.1	82.1	80.8	4.7	106	57	117
14.5	157.6	156.4	159.1	1.5	49.9	51.9	52.3	2.4	78.6	84.2	81.6	3.0	146	23	54
												Together:	702	705	1099

ments of menstruating girls mentioned above, and girls of the similar age-group still not mentruating there is a certain coincidence among the lower values of range. At 13.5 and 14 years old girls, in case of the lower values of the normal circumference of chest, at 13.5, 14 and 14.5 years old girls in case of bicristal breadth, the lower values of range are, in case of menstruating ones lower than at those who are still before the menstruation. In consequence, it is perceivable, that the occurrence of menarche at the girls of the same population cannot be connected exclusively to a definite body weight or stature value as their attainment is related to other factors as well, e.g. race, the level of nutrition, etc. Moreover, only, data derived from longitudinal sampling are worth considering. Finally referring to SCHWENK's note (1965), can be mentioned that the sexual hormones restrain the production of somatotropic hormones. In compliance with it, the menarche heavily depends on the fluctuation of the concentration of oestrogens. We may agree with GRIMM's conception (1966) who claimed that the early-maturing children achieve a higher body development and at the late maturing ones, a so-called leptomorphic tendency of growth can be demonstrated. The estimation of this question requires, certainly, a more detailed analysis in the future. Comparing the arithmetic mean of body height, body weight and normal circumference of chest of all the girls of corresponding age-groups with the similar arithmetic means, from 1958/59 and 1966/67, appears that, the arithmetic mean of 1981 is in every case considerably higher. It occurs only in case of 11.5 years old girls, that the arithmetic means obtained in 1966 and 1981 coincide totally (Table 5). On the contrary according to our experiences at the normal circumference of chest the mean of 1966 is higher than those of 1958/59; on the other hand, the mean values obtained in 1981 did not exceed the results of 1966 either, but they are 1–3 cm below. Considering all these values we must take into consideration that the sample sizes are wery similar (in 1958/59: 702, in 1966: 705 and in 1981: 1099). Therefore the decrease of the mean values of the normal circumference of chest is not connected with the essential difference in sample sizes. The differences cannot be regarded as a methodical error either, as the samplings were carried out by the same person. So, it is indisputable that the process of accelerated growing is in progress even today, as it could be established unambiguously from the comparison of data from 1958/59 and 1966/67, in Szeged. Anyway this was not observed in case of nursery-school children, whose earlier data (FARKAS, IZSÁK and NAGY, 1965) were revised in spring of 1981. The mentioned change of somatic characteristics was followed by the change in the puberty time of girls. While the value of the median of menarche of the girls in Szeged was 13.2 years in 1958/59, 13.02 years in 1961, at the same time it was 12.75 years in 1966/67. The question raises spontaneously, if we can expect a further change of the median size in 1981, too.

In order to establish this, we also performed preliminary evaluation. We have the data of 935 girls, from the age-groups of 11.5–14.5 years, collected with the method of status quo. In 484 cases the menstruation already occured before the date of samplings. The data of observation have been established with a numerical method, applied earlier, which produced a result quite identical with the probit analysis (FARKAS, 1975). Accordingly the menarche age of girls in 1981 in Szeged was 12.72 years, i.e. 0.03 year below the median of an earlier observation. This slight decrease might not indicate a secular trend in the past 15 years; it is, however, a fact that the median didn't shift towards the higher age of life. Our aim is to realize our sampling among the 10 to 18 years age-groups. So conclusions must be drawn very carefully

Table 6. The important values of determination of menarche median.

Age groups x	Total n	The number and the p.c. of menstruated r	%	The probit of p.c. of menstruated P
11.5	153	17	11.1	3.78
12	161	54	33.5	4.57
12.5	150	58	38.7	4.71
13	172	97	56.4	5.16
13.5	128	108	84.4	6.01
14	117	100	85.5	6.06
14.5	54	50	92.6	6.45
	935	484		

The number of series i	Age-groups x_i	Empirical probit y_i	x_i	$x_i y_i$
1	11.5	3.78	132.25	43.47
2	12	4.57	144.00	54.84
3	12.5	4.71	156.25	58.88
4	13	5.16	169.00	67.08
5	13.5	6.01	182.25	81.14
6	14	6.06	196.00	84.84
7	14.5	6.45	210.25	93.53
n=7	91	36.74	1190.00	483.78

as the majority of our data refers to the 11 to 14.5 years age-groups. Thus alteration of this median value, can be expected. It derives from the above mentioned, that the present paper is only the previous evaluation of a large research work. The data obtained so far allow us, however, to suppose — at least in case of the girls in Szeged — that the process of accelerated growing should not yet be considered as finished; which is shown by the significant differences of the arithmetic mean values of body measurements. This fact after all is self-evident, taking into account that Szeged is the centre of a large oil-field, having attracted numerous workers mostly young people from other areas of the country in the last 10–15 years. Thus the mixing up to the population, as well as the urbanization have accelerated. According to our plans, — it should be added — we want to draw into our investigation all the 10 to 18 years old girls of Szeged. Up to the present, however, we could only examine, in the first place, children who live in the new district of about 35 000 inhabitants, whose conditions differ from those living in other areas of the town.

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EFFECT OF NITROUS OXIDE ON THE MITOSIS OF PLANT CELLS

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Abstract

I have established the dependence of the metaphase-blocking effect of N_2O on gas concentration, as well as on the length of the treating period. The highest mitotic activity can be achieved in the cells of the root meristem of rye at 6 atm pressure of N_2O (270 mM concentration) by an 8 hours treatment. The metaphase-blocking effect of N_2O is reversible, following the solution of gas treatment, the metaphase-cells enter into a more or less normal anaphase.

Introduction

In investigating the phenomena of mitosis, a very great role is played by certain cytostatic materials, which hinder the regular process of mitosis. The agents hindering mitosis are called "mitotic poisons" (BIESELE, 1958). As a result of the hindering effect, there may occur specific chromosome-configurations, or polyploidy may be formed.

The mechanism of action of some agents, e.g. colchicine — the alcaloid of *Colchicum autumnale* — and of colchicum derivatives have been studied for long decades. EIGSTI and DUSTIN (1954) established that colchicine brought to stop metaphase. Its effect is so characteristic and specific in mitosis that in literature the designation C-mitosis, colchicine-mitosis, was introduced for this phenomenon (LEVAN, 1938). Apart from colchicine, colcemide has exerted the most marked effect on mitosis (PICKETT-HEAPS, 1967; STUBBLEFIELD and BRINKLEY, 1966; BRINKLEY et al., 1967).

Certain gases (xenon, argon, methane, hydrogen, nitrous oxide) are known to make an effect on the process of mitosis that is similare to that of colchicine. The mechanism of action of these gases has been unknown, so far. The nitrous oxide has produced C-mitosis in pea seedlings under atmospheric pressure (ÖSTERGREN, 1944); on the other hand, in onion a higher pressure was to be applied in order to get similar result (FERGUSON et al., 1950). In 1968, RAO treated mammaliann (HeLa) cells with N_2O and established that the effect of N_2O blocking to metaphase depended on pressure and was reversible. A pressure below 2.72 atmospheres produced no effect on the above mentioned cells. An atmospheric pressure between 2,72–4.42 atm has not caused full inhibition. A full metaphase block could be achieved with a pressure between 5.1 and 5.4 atm and the effect proved to be reversible. The degree of reversibility depends upon the duration of treatment and pressure.

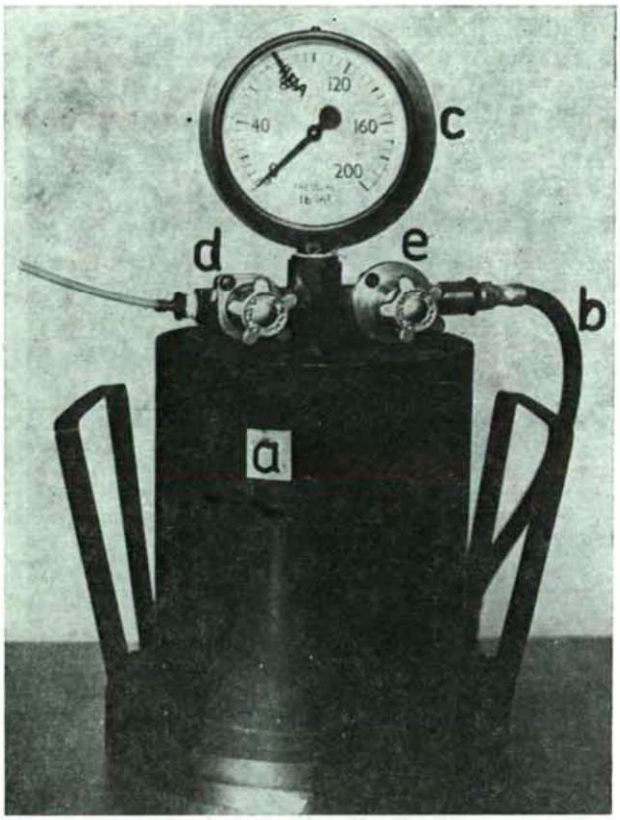

Fig. 1. Pressure chamber used for the nitrous-oxide treatment.
Pressure chamber (a), joint duct (b), manometer (c). The standard pressure in the cylinder was assured by closing both valves (d, e). Finishing the treatment, I decreased the pressure to zero atmosphere by opening the valve (d) slowly.

Similarly to colchicine and colchicine-derivatives, polyploids and aneuploids can be induced by nitrous oxide, as well (ÖSTERGREN, 1954; NYGREN, 1955; ÖSTERGREN, 1957; KIHARA and TSUNEWAKI, 1960; TSUNEWAKI, 1962, DVORAK et al., 1973).

The investigations concerning the effect of nitrous oxide are important from more than one point of view. On the one hand, gas is applied as anaesthetic in the clinical praxis, as well; on the other hand, mode of action of this, exerted on mitosis, is unelucidated, as yet.

Materials and Methods

I have chosen the diploid rye as test plant, having low chromosome number ($2n=14$). Cytological preparations were made from the actively growing root meristems of seedlings. Germination took place at 20 °C, in Petri dishes, on wet filter-paper, in a thermostat, for 20 to 30 hours. Seedlings having three roots — of 6 to 10 mm — were used for experiments. The N_2O treatments were carried out in a pressure chamber, to be seen in Fig. 1, made in the workshop of the University Cambridge. (A gift of Dr. R. T. JOHNSON to the BRC.)

The seedlings were collected for the experiment on a filter-paper in a Petri-dish and put them in a cylinder (a), treating the with N_2O of different concentrations, for a different time.

The plants were fixed in a mixture of absolute alcohol and acetic acid in ratio 3:1, at room-temperature, for 1/2 to 12 hours. The fixed seedlings were stored in 70 per cent alcohol till being used.

The 6 to 10 mm roots of the fixed seedlings were cut, then hydrolized in 1N hydrochloric acid at 60 °C for 16 to 18 minutes and stained in Schiff's aldehyde reagent according to Feulgen's squash method for 40 to 60 minutes.

The comparatively hard and thick plant cell wall renders more difficult making preparations, therefore roots were treated, before being squashed, in non-buffered enzymesolution — containing 2 per cent pectinase (FLUKA, SCHUCHARDT) and 2 per cent cellulase (ONOZUKA R-10, YAKULT) — at room-temperature. In ten minutes, from the softened roots, the enzymesolution was replaced by absolute alcohol. The roots were immersed into concentrated acetic-acid for 1 to 2 seconds and placed on a microscopic slide, and a 2 to 3 mm apical part of roots were cut. After dropping 45 per cent acetic-acid on them the root-tips were covered and squashed. The preparations of suitable quality were fixed with dry-ice method (CONGER and FAIRCHILD, 1953). After freezing, the coverslip was removed and the preparations were dehydrated in alcohol-series, then the slides were immersed into xylene. On the clean coverslips De Pex (G. T. Gurr) was dropped and then the preparations were mounted. The preparations, fixed in this way, can be stored without discolouration and pollution.

The investigations were carried out, and the needed microphotographs made, with a ZEISS NU-2 microscope, on black and white ORWO 15 Din negative film.

The mathematical evaluation of data was carried out the mean of data and the standard error of means were calculated.

The test of homogeneity of variance was carried out with Bartlett's test.

The significance test was performed with a x^2 test, the comparison of arithmetic means with a t-test (SVÁB, 1973).

The obtained results are fixed in a Table and shown in a column diagram.

Results

1) Determination of the gas concentration needed to the optimal inhibition of mitosis (Pressure experiment)

The first question to be answered was, by which nitrous-oxide pressure (concentration) the inhibition of mitosis can be achieved, in case of a treatment of indentical duration. I looked for an answer to, in which degree the mitosis of the root-meristem cells depends upon the N_2O concentration. Seedlings were treated with N_2O under a pressure of 1–12 atm, for two hours. I repeated the experiments three times, on every pressure value, i.e. I investigated 2.000 cells of the root tips of 3 times 3 seedlings in each of the experiments. It was established that, changing the nitrous--oxide concentration, the degree of mitosis-inhibition effect of gas changes, depending upon the pressure. I selected the "optimum" pressure value within the investigated interval, in case of which the highest mitotic activity can be achieved in two hours. The degree of mitotic activity i.e. the ratio of mitotic and interphase cells — considering the length of cell cycle as standard — depends on the effectivity of the inhibition of mitosis. The more effectual the inhibition is, the more mitotic cells are to be found in the seedlings simultaneously.

The inhibition of mitosis manifests itself therein that the mitosis of cells gets only as far as the metaphase. Chromosomes are arranged in a characteristic ring-form (Fig. 2) and the metaphase is not followed by ana- and telophases.

The summary of the experimental data is contained in Table 1. Beyond mitotic activity, I determined the ratio of pro-, meta-, and anaphasic cells at any pressure. The test of homogeneity of the standard deviation, Bartlett's test was used. By

Fig. 2. The "ring" metaphases, coming into existence as a result of nitrous-oxide, at the treatment at 6 atm for two hours (magnification: x1000).

means of this, I established that these do not differ significantly from each other. It was proved by comparing the means with F-test that there is a significant difference between the average mitotic activities of the seedlings, treated under different pressures, at P 1 per cent level. It was controlled with T-test, between the averages of which treatments there is a significant difference and the value of the minimal significant difference was also calculated. In my experiments, this proved to be 1.87 per cent. It is therefore due to the effect of nitrous oxide if in the treatments the mitotic activity differs from the control level by more than 1.87 per cent or if the difference of mitotic activities measured in two treatments is more than 1.87 per cent. It can be established on the basis of significance-investigations that there are significant differences between the treatments of 1 to 3, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 10, 10 to 12 atmospheres. The diagram of mitotic activity — and the ratio of the

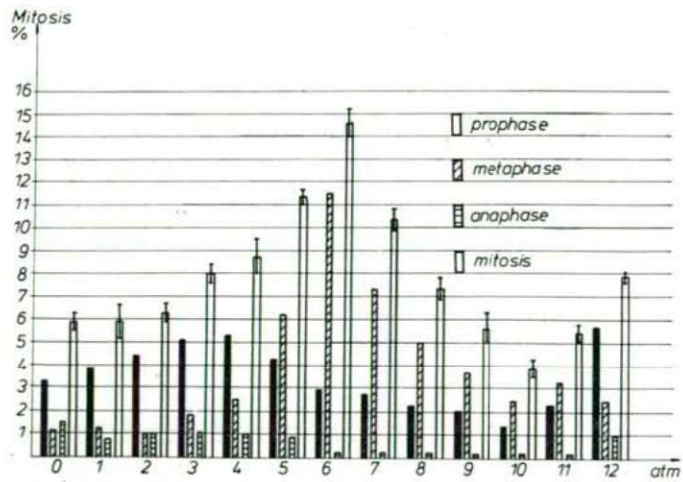

Fig. 3. The pro-, meta-, and anaphase change in the cells of the root meristem, as well as that in the mitotic activity, taken as a function of pressure. Treatment is: 2 hours.

pro-, meta-, and anaphase cells — is to be seen in Figure 3, in which the dynamics of the change in the single mitotic phases can be followed well.

It turns out from the diagram unambiguously that by the change in concentration the ratio of the cells in the mitosis also changes, as compared with the mitotic activity of control cells. A significant inhibition of mitosis (mitotic accumulation) is to be observed, begining from a gas concentration of 3 atm (135 mM). Till atm 6, the number of mitotic cells increases, after this it decreases (at atm 9 to the level of control). At 10 atm, there are fewer cells in mitosis than in the root-tip of the control. This little but significant decrease in the number of mitotic cells is due to the vigorous decrease in the number of prophase cells. At this pressure, the number of prophase cells does not achieve the level of 50 per cent of the control. As this trend ceases to continue after the increase of pressure, and at 12 atm the number of mitotic cells considerably exceeds even the level of control, the effect of the 10 atm pressure cannot be considered as a "saturation".

Analysing the distribution of the phases of mitosis (pro-, meta-, and anaphases), we can conclude that with changing of the gas pressure, the most obvious change follows in the number of metaphases. At 6 atm, the number of metaphase cell increases almost to be 12-fold, as compared with the control. The number of pro phases remains, apart from the decrease mentioned above, approximately on an identical level. Following the formation of anaphases with attention, I have found that the ratio of anaphases shows till a pressure of 5 atm (225 mM gas concentration) a value close to the control. It is, between 6 to 11 atm not more than 10 to 15 per cent of the control, showing unequivocally the full blocking of the metaphase. The final conclusion from the pressure experiment that the highest mitosis accumulation can be achieved at a pressure of 6 atmospheres.

The question arose whether the metaphase-blocking effect of nitrous oxide depends, and in what degree, on the length of the time of treatment.

2) The effect of duration of treatment on the mitotic accumulation (Time experiments)

Seedlings were treated at a pressure of 6 atm and two seedlings were fixed hourly, resp. two-hourly, each, for 1 to 34 hours. The percentage of mitotic, pro-, meta-, and anaphase cells was calculated from a thousand cells, each. The experiment was repeated three-times. The data are summarized in a Table (Table 2).

With Bartlett's test, the investigation the test of homogeneity of the variance were performed. On the basis of mathematical evaluation, the standard deviation of the data of the 20 experiments does not differ significantly from one another. The comparison of arithmetic means was performed with F-test. It is to be established on the basis of the F-test that between the averages of the different treatments there is a obvious significant difference. The single treatments were controlled with t-test in detail and I exactly established between the averages of which groups least significant differences are. I calculated the least significant difference, this proved to be 2.55 per cent. The dependence of the pro-, meta-, and anaphases and of mitotic activities on the time is plotted on column diagram (Fig. 4).

On the basis of analysing the data, it is to be established that the mitotic activity depends on the duration of treatment. The highest mitotic activity was faund at 8 hours treatment (Fig. 5). A treatment longer than 8 hours gradually decreases

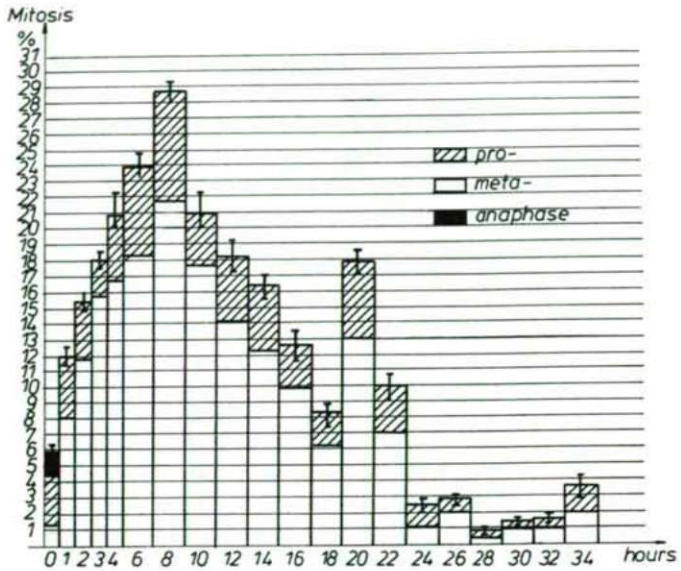

Fig. 4. Change in the mitotic activity of the root-meristem cells, taken as a function of the treatment of time, as a result of a 6 atm gas treatment.

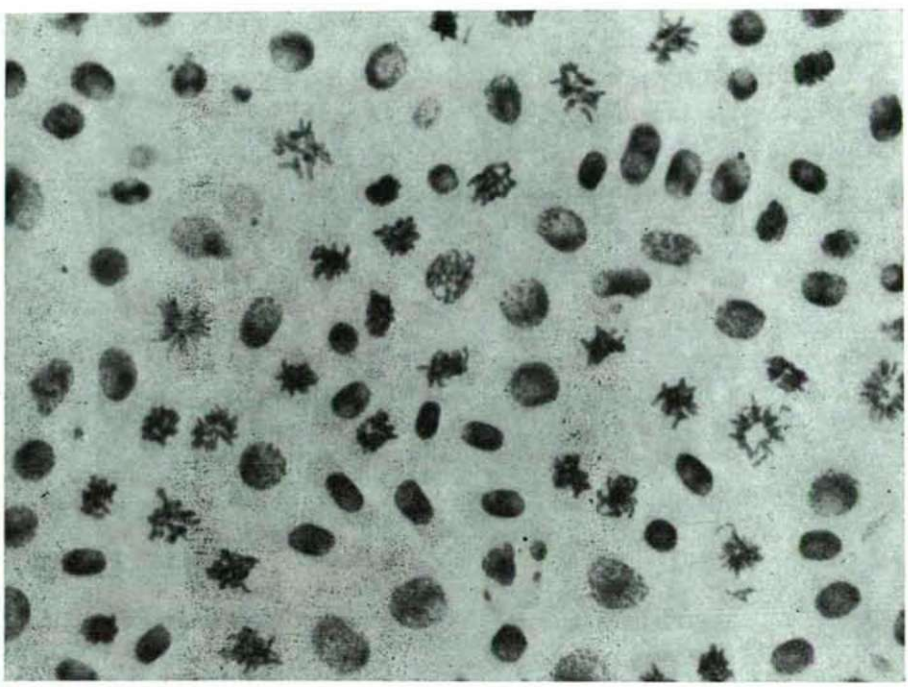

Fig. 5. The effect of an 8-hour nitrous-oxide treatment, at 6 atm pressure, on the division of cells. Magnification: x400.

the number of the cells in mitosis, while the curve reaches its first minimum at 18-hour. It is obvious that this minimum is still higher than that to be observed in the control. At a 20-hour treatment, again a sharp peak can be observed on the curve. The majority of cells at that point are tetraploid. Following this, the mitotic activity begins to decrease steeply till the 20-hour treatment, then it remains below the level of the control all the while.

Studying the given phases of the mitosis, it was faund that after a 2-hour treatment the ratio of anaphases remained on the level of 10^{-4} frequency, i.e. it could be considered as zero. The change in the number of metaphases cells follows the change in the mitotic activity well. With the exception of treatments for 2 and 3 hours the frequency curve of the number of prophase cells is also of like course.

Knowing the nitrous-oxide concentration, blocking the most effective mitosis, as well as the optimum time of treatment, I found it necessary to perform some control experiments for proving that really the specific effect of N_2O is really in question and not the changes by the experiental conditions (high pressure). For the sake of this, at 6 atm pressure, I performed 8-hour treatments with air, nitrogen, and oxigen. At none of these I observed any cytological event, similar to those observed during of the nitrous-oxide treatments. In all the three treatments, mitotic activity remained at control level or — within the limits of significancy — below the level of the control.

3) Deciding the reversible resp. irreversible effect of the nitrous-oxide treatment (Experiment of returning)

In order to decide whether that the effect of nitrous-oxide on mitosis is a reversible process or not I have performed the following experiments. The seedlings were treated at 6 atm pressure for eight hours. Then, after solving the effect of N_2O seedling were fixed in 1/2 resp. 1 hour intervals. The last sample was taken after the termination of gas treatment, in the sample was taken after the termination of gas treatment, in the 21st hour. The experiment was repeated three times. In all the three repeated samples, the percentage of the mitotic, as well as pro-, meta-, and anaphasic cells was counted from 4000 cells, each. The data, obtained from the investigation, are showed in Table 3.

Mathematical control of data, was performed establishing that the standard deviations are not significant, the average values are to be considered as homogeneous. The least significant difference proved to be 2.93 per cent.

The averages of mitotic, pro-, meta-, and anaphasic activities are plotted, like before, in a column diagram, taken as a function of time (i.e. the time following gas solution) (Fig. 6). In the Figure 6 it is to be seen that, after solving gas pressure, mitotic activity decreases abruptly and the anaphase cells immediately appear (Fig. 7).

Following the formation of the certain phases of mitosis, we can see that in 30 minutes after solving gas pressure the number of anaphases rises from zero per cent to 7.3 per cent, then it gradually reaches the control lever. After ten hours, mitotic activity again has an abrupt peak, being almost 2.5 times more than the control value. After ten hours, mitotic activity shows a five-hour periodicity, each presenting itself in the form of a peak, essentially exceeding control level. In these

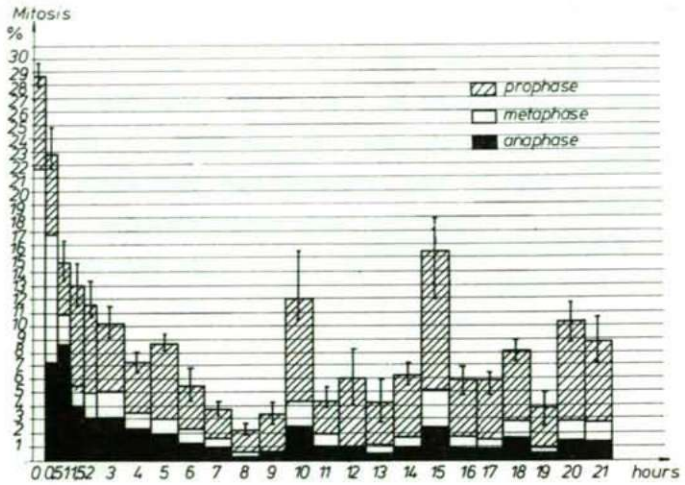

Fig. 6. Change in the pro-, meta-, and anaphase, as well as mitotic activities of the root-meristem cells, taken as a function of the time following gas solution. The treatment lasted at 6 atm pressure, for 8 hours.

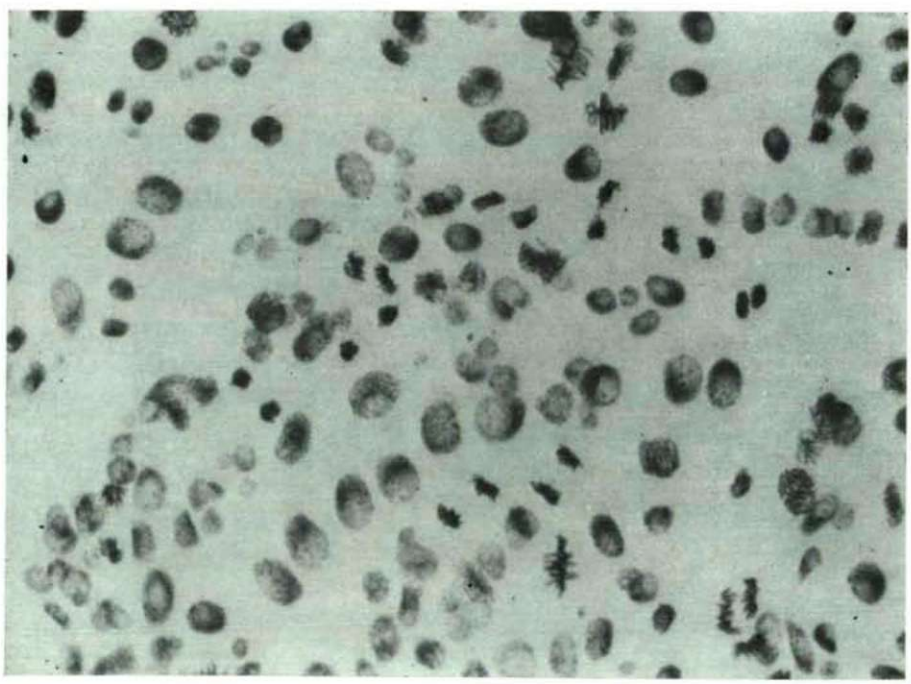

Fig. 7. Change in mitoses, at 6 atm, following the termination of the 8-hour gas treatment. Magnification: x400.

peaks, more than two-thirds of mitotic cells are formed by prophase cells. Apart from peaks, beginning from the 5th hour following gas treatment, the value of mitotic activity does not differ from the control level significantly.

Discussion

At evaluating the effect made by nitrous oxide on the dividing root-meristem of seedlings, it is absolutely necessary to discuss certain questions which are to be considered as peculiarities of the experimental system. For lack of this, the interpretation of experimental results, the conclusions drawn may be the sources of several errors.

It is to be considered as one of the most important questions that *the root-meristem cells cannot be regarded as a homogeneous cell population.* In the root tip, fast-cyclizing cells (of short cell-cycles), slowly cyclizing cells (of long cell-cycles) and non-cyclizing (resting) cells are to be found. It is not yet settled whether these resting cells are only in a provisional resting state or they remain definitely "out of the cell cycle". For the time being, there is no direct evidence for that these cells have lost their mitotic activity (CLOWES, 1976). It is obvious that the existence of these three basic cell types makes extremely complicated to evaluate any effect and this fact is absolutely to be had in sight. At elaborating the results, therefore, we should leave out of consideration every evaluation supposing a uniform cell cycle (i.e., some cells dividing with identical speed, identical rate). The root-tip preparations made in the course of investigations consisted on average number of 3500 to 4000 cells. In the certain experiments, we evaluated uniformly 2000 cells, each, which is approximately 50 per cent of the full preparation. I should have liked to decrease to minimum the errors given by the different "answers" of "fast", "slow", "non-dividing" cells.

Table 1. Determination of the gas concentration, needed to inhibit the optimum mitosis (Pressure experiment). Average values of the pro-, meta-, and anaphases, as well as of mitotic activities, and the dispersion values of the arithmetical average of mitocic activities. (The time of the nitrous-oxide treatment is two hours at each pressure.)

Mitosis N_2O pressure (atm)	Prophase	Metaphase	Anaphase	Mitosis	$s_{\bar{x}}$**
Control	3.32	1.09	1.48	5.89	0.41
1 atm	3.79	1.24	0.84	5.87	0.79
2 atm	4.40	0.96	0.98	6.34	0.43
3 atm	5.07	1.84	1.06	7.97	0.39
4 atm	5.28	2.50	0.92	8.70	0.80
5 atm	4.19	6.18	0.89	11.26	0.34
6 atm	2.93	11.35	0.23	14.51	0.57
7 atm	2.74	7.34	0.24	10.39	0.49
8 atm	2.16	4.98	0.15	7.29	0.51
9 atm	1.97	3.61	0.06	5.64	0.57
10 atm	1.32	2.39	0.12	3.83	0.39
11 atm	2.17	3.16	0.11	5.44	0.28
12 atm	5.58	2.40	0.86	8.84	0.20

** $s_{\bar{x}}$ Standard deviations of the averages of mitotic activities.

Using 2-hours experimental times during the pressure experiments (Table 1, Fig. 3), I have established that within the investigated series of 1–2 atm pressure intervals, *a mitotic accumulation* can be achieved at a pressure of 6 atmospheres. The accumulating effect of the nitrous-oxide mitosis can unambigously be explained by increasing the number of metaphases, i.e. by metaphase block of cell division. Owing to technical causes (lack of an apparatus of high pressure), I could not investigate into the repeated rise of the mitotic activity at 12 atmospheres. Thus, I cannot give any satisfactory explanation to this. After establishing the "optimum" gas concentration (6 atm), I investigated into the effect of the time of treatment on the mitotic accumulation, within an interval of 1 to 34 hours (Table 2, Fig. 4). The number of mitotic cells is roughly doubled by only 1-hour treatment at 6 atm pressure, this being the result of an about 7-fold increase in the number of metaphase cells, as compared with the control. The highest mitotic accumulation was achieved at the treatment of 8 hours, where the ratio of metaphase cells was about 20-fold, comparing with the control.

There is to be found more than one explanation for the rise in the number of prophases, namely: 1) The prophase phase relatively lengthens, while the time of cell cycle remains unchanged. 2) The cell cycle accelerates, 3) mitosis-induction in the non dividing cell population, etc. The number of metaphases gradually decreases after a treatment for eight hours, from which the conclusion can be drawn that the metaphase-blocking effect of the nitrous-oxide gas is restricted in time and, after a treatment exceeding the 8 hours, already more cells go over to an interphase,

Table 2. Effect of the time of treatment on the mitotic accumulation (Time experiment). Average values of the pro-, meta-, and anaphases, as well as of mitotic activities, and the dispersion values of the arithmetical average of mitotic activities. (The nitrous-oxide treatment took place at six atmospheric pressure.)

Mitosis Time (hrs)	Prophase	Metaphase	Anaphase	Mitosis	$s_{\bar{x}}$**
Control	3.32	1.09	1.48	5.89	0.41
1	3.53	8.10	0.05	11.68	0.63
2	3.18	11.88	0.28	15.34	0.72
3	2.35	15.67	0.09	18.11	0.40
4	4.15	16.57	0.00	20.72	1.58
6	5.42	18.23	0.12	23.77	1.03
8	6.83	21.70	0.03	28.56	0.58
10	4.78	17.63	0.03	22.44	1.47
12	4.12	14.07	0.07	18.25	0.96
14	4.08	12.23	0.00	16.31	0.68
16	2.55	9.92	0.02	12.49	1.12
18	2.00	6.25	0.00	8.25	0.53
20	4.80	12.97	0.00	17.77	0.59
22	3.03	6.98	0.00	10.01	0.83
24	1.32	1.27	0.00	2.59	0.30
26	0.80	2.08	0.00	2.88	0.33
28	0.28	0.57	0.00	0.85	0.14
30	0.60	1.00	0.00	1.60	0.22
32	0.67	1.05	0.00	1.72	0.26
34	1.72	2.10	0.00	3.82	0.59

** $s_{\bar{x}}$ Standard deviations of the averages of mitotic activities.

omitting the ana- and telophasic phases than the number of cells going newly into mitosis. After 20 hours, the number of metaphase again shows a rise. But most of the mitotic cells are after twenty hours are tetraploid. The peak is, therefore, given not by cells stepped into a "new" mitosis, but already "treated" cells get again till the metaphase. From this, the conclusion can be drawn that:

1) by nitrous oxide, no notable inhibition is induced in another stage of the cell cycle,

2) during the 6 atm nitrous-oxide treatment, the cycle time of these cells is approximately 10 hours. A treatment shows after 22 hours an anequivocal inhibitory effect and till a treatment for 34 hours I have not observed any following rise. Beyond the inhibitory effect induced by the long treatment, a part can be played in this by the exhaustion of dividing ability of the cells already divided.

With control experiments, I established that *the effect of nitrous oxide on the mitosis is specific*, and it is not due to the physical conditions of the treatment (e.g. high pressure). 6-atm, 8-hour treatments were performed with air, nitrogen, and oxygen and none of these resulted in an effect, similar to that of N_2O.

Table 3. Decision on the reversible resp. irreversible effect of the nitrous-oxide treatment. (Experiment of returning.)
Arithmetical averages and dispersion values of the pro-, meta-, and anaphases, as well as of mitotic activities.
(Nitrous-oxide treatment at 6 atm pressure, for 8 hours, sampling following gas solution, in different points of time.)

Mitosis Time (hrs. min.)	Prophase	Metaphase	Anaphase	Mitosis	$s_{\bar{x}}$ **
Control	3.32	1.09	1.48	5.89	0.41
0.00	6.84	21.70	0.03	28.60	0.58
0.30	5.80	9.51	7.28	22.78	0.99
1.00	3.85	2.22	8.60	14.67	0.75
1.30	7.33	1.53	4.13	12.97	0.83
2.00	6.48	2.13	3.00	11.60	0.94
3.00	4.90	2.10	3.17	10.17	0.55
4.00	3.68	1.32	2.30	7.30	0.32
5.00	5.17	1.17	1.86	8.72	0.33
6.00	3.13	1.07	1.30	5.50	0.59
7.00	2.18	0.72	1.03	3.93	0.24
8.00	1.57	0.53	0.22	2.32	0.18
9.00	2.28	0.60	0.58	3.47	0.44
10.00	7.10	1.90	2.47	11.97	1.72
11.00	2.47	1.02	0.92	4.40	0.45
12.00	4.15	0.93	1.05	6.13	0.98
13.00	3.02	0.88	0.40	4.30	0.92
14.00	4.63	0.87	0.78	6.28	0.51
15.00	10.22	2.83	2.35	15.40	1.68
16.00	4.12	0.97	0.72	5.80	0.46
17.00	4.33	0.83	0.67	5.83	0.29
18.00	5.07	1.33	1.63	8.03	0.43
19.00	3.08	0.38	0.38	3.85	0.59
20.00	7.43	1.50	1.28	10.22	0.65
21.00	5.87	1.28	1.43	8.58	0.92

** $s_{\bar{x}}$ Standard deviations of the averages of mitotic activities.

According to my investigations, *the effect of nitrous oxide is reversible*. Following the solution of the gas treatment, the metaphase cells immediately pass over into anaphase.

After solving the gas treatment (Table 3), the change in *mitotic activity shows a definite periodicity*. For 9 hours, mitotic activity gradually decreases, then, at 10 hours, it achieves a level doubling that of the control, and followed after 15 resp. 20 hours again by a new peak, in each case.

On the data of the time experiment, it is not probable that this phenomenon would be a consequence of a partial blocking, taking place at some point of the cell cycle. I consider as a more probable explanation that the 10-hour peak is induced by the fast cyclizing cells partially remaining in synchronized, while the 15-hour peak is formed by the cells of the cell population of a faster cell cycle, remaining in synchron. The 20-hour peak is again produced by the fast cyclizing cells of supposedly 10-hour cell cycle. On the basis of this explantion, the larger share of the nearly 30 per cent mitotic cell population of zero hour zero minute would be given by the slower cells (of 15-hour cell cycle). The sum of the 10- and 15-hour peaks agrees extremely well with the mitotic activity observed at the termination of gas treatment. By this, as well as by the comparison of the 10- and 20-hour mitotic activities, the extremely high stability of synchronity is shown.

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